PB-233 599

ESTIMATION OF POLYCHLORINATED BIPHENYLS IN THE PRESENCE OF DDT-TYPE COMPOUNDS

J. T. Brownrigg, et al

Baird-Atomic, Incorporated

Prepared for:

Environmental Protection Agency

June 1974

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TECHNICAL REPORT DATA

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1. REPORT NO. EPA-670/4-74-004
2.
3. PB 233 599

4. TITLE AND SUBTITLE
Estimation of Polychlorinated Biphenyls
in the Presence of DDT-type Compounds

5. REPORT DATE
June 1974; Issuing Date

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
J.T. Brownrigg and A.W. Hornig

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Baird-Atomic, Inc.
125 Middlesex Turnpike
Bedford, Massachusetts 01730

10. PROGRAM ELEMENT NO. (16202GIY)
1BA027;ROAP 09ABZ;TASK013

11. CONTRACT/GRANT NO.
68-01-0082

12. SPONSORING AGENCY NAME AND ADDRESS
National Environmental Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

13. TYPE OF REPORT AND PERIOD COVERED
Final Report, February 1974

14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

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INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

16. ABSTRACT

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The present procedure involves collection of grab samples followed by extraction, drying, concentration, and redilution in a second solvent suitable for luminescence measurement at 77°K. Studies include the determination of recoveries and detection sensitivities for some of the compounds of interest and also analyses of several environmental waters.

Detection limits for p,p'-DDT and Aroclor 1254 doped in 1-liter samples of pure water were found to be approximately 0.5 and 0.03 ppb respectively. Sensitivities were reduced by an order of magnitude or more in natural waters having high levels of dissolved organic material and particulates. This is due to a combination of poorer recoveries and increased fluorescence background. Both of these remain as problem areas deserving further study. Phthalic acid esters have spectral features resembling certain Aroclors and may constitute an interference.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
*DDT, *Fluorometers, Spectroscopy, *Water analysis, Aromatic compounds, Chemical analysis, Water pollution, Chlorine aromatic compounds, Polyphenyl compounds, Luminescence	*Aroclor, *Analytical techniques, *Pollutant analysis, Chlorinated hydrocarbon pesticides, *Polychlorinated biphenyls, *Low temperature luminescence	7C 13B

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 100
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE $4.00

PA Form 2220-1 (9-73)

☆ U.S. GOVERNMENT PRINTING OFFICE: 1974— 757-584/5324

EPA-670/4-74-004
June 1974

ESTIMATION OF POLYCHLORINATED BIPHENYLS
IN THE PRESENCE OF DDT-TYPE COMPOUNDS

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Program Element No. 1BA027 (16020 GIY)

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CINCINNATI, OHIO 45268

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The National Environmental Research Centers provide this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere,
- the development of efficient means of monitoring these contaminants, and
- a search for ways to prevent contamination and to recycle valuable resources.

The investigation reported herein was conducted for the National Environmental Research Center--Cincinnati to explore the use of a new technique for the identification and measurement of polychlorinated biphenyls in the presence of similar organic compounds. The study established the experimental conditions necessary for the detection of PCB compounds, the sensitivity of the determination, and the applicability of the method to natural water examination.

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Director
National Environmental
Research Center, Cincinnati

ABSTRACT

Earlier studies suggested that the low temperature luminescence properties of PCB's and DDT compounds could be used to identify these compounds singly or in mixtures. The present investigation was undertaken to develop a relatively simple, rapid method for estimating these compounds in water. The emphasis in this procedure has been on the inherent sensitivity and specificity of luminescence, avoiding chemical separation where possible.

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Detection limits for p,p'-DDT and Aroclor 1254 doped in 1-liter samples of pure water were found to be approximately 0.5 and 0.03 ppb respectively. Sensitivities were reduced by an order of magnitude or more in natural waters having high levels of dissolved organic material and particulates. This is due to a combination of poorer recoveries and increased fluorescence background. Both of these remain as problem areas deserving further study. Phthalic acid esters have spectral features resembling certain Aroclors and may constitute an interference.

This report was submitted in fulfillment of Program Element No. 16020 GIY and Contract/Grant No. 68-01-0082 by Baird-Atomic, Inc. under the sponsorship of the Environmental Protection Agency. Work was completed as of February, 1974.

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ACKNOWLEDGMENTS

The authors would like to thank Mr. D. G. Ballinger of the Methods Development and Quality Assurance Research Laboratory, National Environmental Research Center, U.S. Environmental Protection Agency, Cincinnati, Ohio, for his interest and encouragement.

Samples of Aroclors and related technical information were supplied by Mr. W. B. Papageorge and Dr. E. S. Tucker of the Monsanto Company, St. Louis, Missouri, and their assistance in this regard is greatly appreciated.

We particularly wish to thank Dr. Bonnie Dalzell, Mrs. Geraldine Garnick, and Mrs. Geraldine Wiesen for valuable technical assistance with the laboratory studies and with the preparation of this report.

SECTION I

CONCLUSIONS

1. The present study indicates that, using an extraction procedure combined with luminescence measurement at 77°K, PCB's (Aroclors) and DDT derivatives can be determined at sub-part per billion levels in water. Detection sensitivities for DDT-type compounds are poorer than for Aroclors because of inherently weaker phosphorescence intensity.

2. Detection limits for PCB/DDT in natural water samples having high levels of fluorescent materials and suspended particulates are more than an order of magnitude worse than in pure water. These limitations could probably be removed by the inclusion of a simple chromatographic step and/or the use of improved extraction techniques.

3. Exploratory studies of some phthalic acid esters (phthalates) show that these compounds are phosphorescent at low temperature. The spectral features of these compounds resemble those of certain Aroclors and might therefore constitute an interference.

4. The method described, which utilizes commercially available instrumentation, should be useful as a screening method in either a mobile laboratory or small field station.

5. Although the procedure developed here does not include coupling with either gas or liquid chromatography, low temperature luminescence measurement could in principle be used profitably with either procedure. In the simplest procedure, specific effluents (or groups of effluents) could be analyzed for confirmatory measurements. Ultimately, it would be very desirable to develop a low temperature luminescence detector to be used in conjunction with a gas chromatographic method.

6. The method developed in this study is, in principle, extensible to other commercial instrumentation. The double monochromator instrument used in this study is particularly applicable to the high-scattering geometry

inherent in low temperature dewar systems. Single monochromator instruments are expected to have higher optical efficiency. It is difficult to predict whether overall detection sensitivity will be increased or decreased because of the offsetting effects of higher efficiency and increased scatter.

SECTION II

RECOMMENDATIONS

The recommendations for future work involved both the study of improved collection/extraction methods and means of minimizing interferences.

1. Recently developed extraction techniques employing polymer resins should be evaluated for compatibility with low temperature luminescence (LTL) analysis. Since much larger volumes of water can be processed, detection limits may be substantially reduced.

2. Means of adapting the present method to suspended particulates should be investigated, since substantial amounts of water pollutants may be solublized or transported in this form. For the same reasons, possible extension to the analysis of bottom sediments should also be considered.

3. Simple separation steps, such as silica gell chromatography, should be tested for ability to remove interfering luminescent substances present in natural water. This would result in greatly improved detection sensitivities for PCB/DDT compounds.

4. Spectra of commonly used phthalic acid esters (phthalates) should be further documented, and their phosphorescence lifetimes measured. Preliminary studies indicate that these compounds have spectra resembling those of certain Aroclors, and therefore may constitute an interference. However, the phosphorescence lifetimes may be substantially longer than those of highly chlorinated PCB's and this might permit temporal separation.

5. Low temperature luminescence spectra of a wide variety of aromatic compounds should be obtained, preferably in a common solvent. These data would be of help not only to establish possible interferences for PCB/DDT compounds, but would be useful for purposes of compound identification or confirmation. Compounds studied initially should be those which have been identified in water using other methods, such as gas chromatography/mass spectroscopy.

6. Additional studies are needed to better establish the accuracy and precision of the present method using a greater number (and variety) of water samples. The reproducibility of the low temperature luminescence measurement could probably be improved by careful selection of the sample tubes (to assure uniformity) and by rapid rotation of the sample tube. The efficiency of the extraction procedure would best be determined using an independent analytical technique such as gas chromatography.

SECTION III

INTRODUCTION

In an earlier phase of the present program, low temperature luminescence (LTL) spectra of several PCB isomers and mixtures (Aroclors) were compared with spectra of some DDE and DDT derivatives. This study also included the analysis of several Aroclor/DDT mixtures, and indicated that PCB's could be identified in the presence of DDT derivates by utilizing inherent differences in the excitation and emission spectra.

The present effort has been directed principally at the development of a simple method for the estimation of PCB's and DDT in water samples. A secondary objective has been to determine limitations of the method and to consider possible coupling of LTL analysis with existing chromatographic methods.

The procedure employed in the present program consisted of collecting grab samples of water, extracting these with dichloromethane to remove organic material, concentrating the extracts, and finally adding a second solvent (methylcyclohexane) suitable for LTL measurement.

This report begins with a brief discussion of molecular luminescence, emphasizing empirical rather than theoretical concepts. This is followed by a discussion of the LTL spectra and detection sensitivities for several Aroclors and DDT derivatives in methylcyclohexane.

Analytical techniques are described for both pure water and natural (river) water samples doped with Aroclor 1254 and p,p'-DDT. Recoveries and detection sensitivities are also determined. Finally, analyses of environmental samples are discussed with respect to PCB/DDT and other luminescent substances present in natural waters.

SECTION IV

LOW TEMPERATURE EMISSION SPECTROSCOPY

Most of the luminescence data appearing in this report were obtained with the sample cooled to liquid nitrogen temperature (77°K). There are several advantages to working at this temperature, and these will be discussed briefly here. Of greatest importance to the present study is that the strongest emission from PCB's and DDT derivatives is phosphorescence, which appears only at low temperature.

Phosphorescence results from the return of a molecule in a triplet electronic state (usually the lowest) to the ground state. In this respect it differs from fluorescence, wherein molecular emission occurs between an excited singlet state and the ground state. These electronic states are shown schematically in Figure 1, where S_0 represents the ground state, S_1 and S_2 are excited singlet states, and T_1 and T_2 are triplet states. (Note: Figures are grouped at the end of the main text, beginning with page 47.) Intersystem crossing is a non-radiative process by which triplet states are populated following absorption to singlet states.

In a given molecule showing both fluorescence and phosphorescence, the phosphorescence will occur at longer wavelengths and will show a much longer lifetime. The lifetime, or decay time, is defined as the time required for the emission to fall to 1/e of its original intensity upon terminating excitation. Phosphorescence lifetimes are generally in the range of one millisecond to several seconds and can usually be measured using mechanical choppers. Fluorescence lifetimes, however, are generally in the nanosecond range and measurement requires much more sophisticated equipment. Lifetime measurement is thus another spectral parameter which may be used for compound characterization.

The longer lifetimes of triplet molecules make them subject to non-radiative deactivation (quenching) by collisions with solvent molecules, oxygen, and other species. Imbedding the phosphorescent molecules in a solvent matrix at low temperature reduces the probability of these quenching processes, enabling observation of phosphorescence. Although fluorescence is less susceptible to such quenching, lowered temperatures may also enhance the fluorescence intensity.

As mentioned previously, chlorinated (and in fact, halogenated) aromatics generally have lower fluorescence yields, but higher phosphorescence yields, than the parent hydrocarbon. Halogenation also shortens the phosphorescence lifetime (McClure, 1949). The mechanism responsible for this is called the "internal heavy-atom effect." Basically, addition of halogen substituents favors the rate of triplet population by depletion of excited singlet states. Molecules of interest here offer good examples of this effect. Biphenyl shows approximately equal yields of fluorescence and phosphorescence, where chlorinated biphenyls show phosphorescence/fluorescence yields more than 100 times greater (Dreeskamp et al., 1972). Also, isomers of DDT and DDT show moderately strong phosphorescence, but fluorescence of these molecules is at least 100 times weaker (Brownrigg et al., 1972; hereafter, this work is referred to as the "first report").

Another advantage of low temperature vs. room temperature analysis is that fine structure may appear at low temperature. This fine structure represents vibrational transitions accompanying the electronic transition. In an emission spectrum, vibration intervals characteristic of the ground state would be observed, and certain of these vibrations would also be observed in the infrared or Raman spectra.

The amount of vibrational structure observed varies with both the particular molecule and the choice of solvent. Usually solvents which freeze to an ordered crystalline state produce sharper structure than glassy (amorphous) media. This is probably because the crystalline medium allows only a small number of orientations available to the guest (emitting) molecule, whereas a great number of random orientations are possible in the glass.

The use of polycrystalline n-alkane matrices to produce these so-called "quasi-line" spectra was pioneered by Shpolskii (1960-1963). Sharpest spectra are usually obtained using solvents having molecular dimensions nearly the same as those of the guest molecule, and spectral bandwidths decrease strongly with decreasing temperature. As an example of this effect, Figure 2 shows the spectra of biphenyl in heptane at both room temperature and at 77°K (note the absence of phosphorescence at room temperature). Also included is a spectrum of biphenyl in methylcyclohexane (MCH) at 77°K. Heptane freezes to a highly scattering "snow," whereas MCH forms a glass.

The scattering properties of heptane would probably make it a less desirable solvent for quantitative work. Note, however, the more highly structured emission obtained in heptane; this suggests the desirability of heptane for analysis requiring higher sensitivity and selectivity for biphenyl.

Unfortunately, the more highly chlorinated PCB isomers and DDT derivatives discussed in the first report show unstructured emission in both MCH and heptane, so that there is apparently no advantage to be gained from a Shpolskii-type solvent in this case. Since the PCB's most likely to be found in the environment are of this type (highly chlorinated), the solvent selected was MCH. As mentioned above, this solvent freezes to a clear, rigid glass, resulting in less scattered light.

To summarize, there are several advantages to be gained from luminescence measurement at low temperature. First, halogenated aromatics (such as PCB's and DDT) exhibit moderately strong phosphorescence, but much weaker fluorescence. Phosphorescence, however, is ordinarily not observed at room temperature. Other molecules which also show fluorescence will often show enhanced fluorescence emission at low temperature. Depending upon the particular molecule and the solvent chosen, lowered temperatures may produce more highly structured absorption and emission; this in turn enables both greater sensitivity and selectivity.

SECTION V

AROCLOR-PESTICIDE SOLUTIONS IN METHYLCYCLOHEXANE

Spectra of several Aroclors and DDT-type compounds, both singly and in mixtures, were studied in methylcyclohexane at 77°K. The purpose of these studies was to obtain basic spectral signatures for comparison with natural water luminescence signatures. These studies also provide a knowledge of the concentration range where response is linear, and an estimate of ultimate sensitivity and selectivity.

In the first report it was noted that the spectra of the o,p'- and p,p'-isomers of DDD and DDT were all very similar. Also emissions from DDE were found to be very much weaker than that of DDD or DDT. For these reasons, only p,p'-DDD and p,p'-DDT have been included in the present study.

The PCB's most likely to be found in environmental waters have isomer distributions resembling commercial mixtures such as Aroclor 1248 and 1254. Therefore, these two Aroclors, plus the newer product Aroclor 1016, have been included in the present study. Aroclor 1016 has a composition similar to Aroclor 1242, but with isomers having five or more chlorine atoms removed (Nisbet and Sarofim, 1972).

Sources

Pesticide samples were obtained from the EPA Perrine Primate Laboratory, Perrine, Florida. Samples were designated "Reference Standard" and had stated purities of 99+%. These compounds were used as received.

Aroclor samples were obtained from Dr. E. S. Tucker and Mr. W. B. Papageorge of the Monsanto Company and were used as received.

Choice of Solvents

For quantative determinations, it is important that the solvent chosen should freeze in a uniform and reproducible manner. Most organic solvents would probably form clear crystals if cooled slowly enough, but this slow cooling would not be practical for rapid analyses. Also, unless the solute can easily occupy the solvent crystal lattice, the slow rate of cooling would probably favor the rejection of the solute from the lattice. (Fractional freezing is in fact sometimes used for solvent purification.) Thus, rapid freezing is usually necessary but in turn will often result in a highly cracked, opaque polycrystalline mass having the appearance of snow. Most common organic solvents such as hexane, carbon tetrachloride, benzene, and acetone exhibit this behavior. This type of crystal matrix is generally undesirable for luminescence analysis for several reasons. Local solute concentrations can be highly variable depending on the rate of freezing. Macroscopic cracks often develop which can influence both the luminescence intensity and the background scatter. This gives rise to variability in luminescence intensity and also contributes a large background signal from scattered exciting light.

Another important consideration, which should be more obvious, is that the solvent itself not be strongly absorbing or emitting at the analytical wavelengths. Thus benzene and its derivatives, which begin to absorb at wavelengths of 270 nm or longer (and emit themselves) would usually be undesirable solvents.

For these reasons the solvent chosen should be non-aromatic and should freeze to a clear, rigid glass, but only a small number of solvents meet these requirements. Winefordner and St. John (1963) have evaluated many solvents and solvent mixtures for ability to form glasses, and an expanded tabulation has been given by Winefordner, McCarthy, and St. John (1967). Westrum and McCullough (1963) also include glass-forming compounds in their tabulation of thermodynamic data for organic compounds.

We have had particularly good success with methylcyclohexane (MCH). The PCB/DDT compounds are soluble at relatively high concentrations (at least 100 ppm), and the solvent itself is non-absorbing and non-emitting; ultraviolet absorption begins at about 230 nm. Sample concentrations of at least 100 ppm can be frozen quickly (15-30 seconds) to liquid nitrogen temperature without cracking. Our experience thus seems to contradict the results of Winefordner and St. John (1963), who consider MCH "not usable" due to high frequency of crack formation. The cracking which we occasionally observe is believed to result from residual water remaining after sample tubes have been washed.

Commercial grades of methylcyclohexane usually contain luminescent impurities. The most prevalent of these impurities appears to be toluene, which has a structured fluorescence in the 270-320 nm region and a partially structured phosphorescence in the 350-500 nm region. Because the excitation spectrum is in the 240-270 nm region, it would interfere with the determination of DDT-type compounds and, to a lesser extent, with PCB's.

The toluene concentration of commercial MCH ranges from less than 1 to over 1000 ppm. Practical or technical grade solvents have the highest toluene concentrations and would not be particularly suitable without purification.

Solvent Purification

Methylcyclohexane--The solvent used for most of this work was Matheson, Coleman, and Bell Spectroquality grade. This solvent typically contains about 0.3 ppm toluene. The second monthly report erroneously reports that this solvent contains about 5 ppm toluene. This value was obtained by comparison of the intensity with a 500 ppm standard, which was later found to be too strongly absorbing to enable a linear extrapolation. The resulting phosphorescence intensity is comparable to that of PCB/DDT when the latter are presented at concentrations on the order of 0.1 ppm. Therefore standard solutions containing such low concentrations are best done using toluene-free solvents.

Originally, efforts focused on purification of practical grade MCH, which is much cheaper than the Spectroquality material. The toluene concentrations, however, are in the 1000 ppm range, making it unsuitable for most luminescence work.

Various procedures were used in an attempt to purify the practical grade material: distillation, sulfuric acid wash, and column chromatography using silica gel and activated carbon. None of these procedures reduced the content appreciably. It is possible that no treatment, except possibly exhaustive hydrogenation, can appreciably reduce high levels of toluene. However, it was found that silica gel chromatography was effective in removing the much lower levels of toluene present in the Spectroquality material. This procedure is simple, efficient, and fairly rapid. A more elaborate variation of this technique was first developed by Potts (1952).

Woelm silica gel, activity grade I (200 mesh) was first Soxhlet extracted overnight with pure dichloromethane. This extraction removes fluorescent contaminants which may be present. The extracted material was then baked overnight in an oven at 160-180°C.

A one-inch diameter chromatographic column with Teflon stopcock was filled to a depth of about 30 cm with silica gel. The silica gel was added as a slurry in MCH with occasional tapping to free air bubbles. The level of MCH was kept above the silica gel at all times.

Methylcyclohexane (Matheson Spectroquality) was allowed to pass through the column at rates on the order of 1 ml/min. After discarding the initial 75 ml, five 100 ml fractions were collected.

Luminescence analysis of the various fractions at 77°K showed no contaminants other than trace amounts of the toluene present in the starting material. The first three fractions appeared to be the best, and contained at least thirty times less toluene than did the starting material (which contained about 0.3 ppm toluene). The fourth and fifth fractions contained about twice as much toluene as the preceding fractions, but were still about a factor of ten better than the starting material.

Dichloromethane (Methylene Chloride)--This solvent, obtained from Fisher Chemical Company as the "Spectranalyzed" grade, was usually found suitable for use as received, but on one occasion was found to contain high concentrations of luminescent impurities.

Impure dichloromethane was purified by distillation, the fraction boiling at 42°C being retained.

Spectra of Pesticides, Aroclors, and Their Mixtures

All emission/excitation spectra appearing in this report were obtained on a Baird-Atomic Model SF-100 "Fluorispec" fluorescence spectrophotometer, using standard optics and accessories. Optically, this instrument is equivalent to the earlier Model SF-1, but has improved electronics. Both instruments employ a 150 watt xenon source, with light dispersed by dual monochromators for both excitation and emission. These instruments are single beam types, and the excitation/emission spectral intensities are uncorrected for instrument response. Although corrected excitation and emission spectra could be produced if desired, the present method, being based on comparisons with standards, does not require these corrections. A good discussion of how these corrections may be obtained is given by Parker (1968).

The first report contains many spectra obtained on the SF-1. Small differences in grating and mirror efficiency and phototube response characteristics give rise to spectral differences obtained with the two instruments. Since the SF-100 was used exclusively for the present study, some of the more important pesticide and Aroclor spectra have been repeated.

Wavelength Calibration--The wavelength calibration of the SF-100 was assumed accurate to ±2 nm over the standard range of 220-700 nm. Unfortunately, after much of the present work was completed, a check of the instrument revealed that the wavelength calibration was beyond accepted tolerances in certain spectral regions.

The accuracy of the emission monochromator was checked by comparison of the dial settings with the discrete spectrum from a low pressure mercury lamp. Wavelengths of mercury lines may be found in the AIP Handbook (Crosswhite, 1972). Excitation wavelength corrections were obtained by scanning the excitation monochromator through the scatter peak at a given fixed emission wavelength, then applying the appropriate correction for the emission wavelength as obtained previously.

Curves showing the wavelength correction factors as a function of the apparent (dial) wavelength are given in Figure 3. To obtain corrected wavelengths, the appropriate correction factor is subtracted from the apparent wavelength. Wavelengths indicated on all spectra in this report represent actual dial readings and are thus uncorrected.

Excitation spectra of the pesticides and PCB's studied here are strongest in the 240-290 nm region, and the wavelength correction factors are relatively small (0-3 nm). Also, the phosphorescence emissions of these same compounds are strongest in the 380-500 nm region, and here the correction factors are within the expected tolerances (2 nm or less). Correction factors for excitation become quite large for wavelengths of 300 nm or longer, where there is significant absorption by natural substances in water.

The loss of acceptable wavelength calibration in our instrument was quite unexpected and probably resulted from its previous shipments to field locations and associated rough handling. It is advisable that the monochromator wavelengths be checked occasionally (e.g., every six months), particularly if the instrument has undergone shipment. In particular, it must not be assumed that the curves in Figure 3 are representative of every SF-100, since a standard instrument should be correct within ±2 nm.

Instrumental Parameters--Certain instrumental parameters which remained largely unchanged have been omitted from the labels on the spectra. Thus, the Hewlett-Packard X-Y recorder gain was typically 5mv/cm, and the photomultiplier voltage (RCA 1P28 tube) was 750 volts. The SF-100 time constant was always 0.3 seconds, and the slow wavelength scan mode (1 nanometer per second) was used exclusively. When these parameters differ, this has been noted on the spectrum label.

The instrumental slit widths employed were usually 22/11 or 33/11 for emission spectra, and 11/22 or 11/33 for excitation spectra. The two numbers on either side of the slash are mechanical positions of slit controls, referring to either the excitation (left pair of numbers) or emission (right) monochromator. There are two slit controls for each monochromator and three possible positions for each control. In particular, the combinations 11, 22, and 33 give spectral bandwidths of about 2, 5, and 26 nm respectively. Our preferred approach has been to use the narrowest set of analyzing slits in order to obtain the optimum spectral resolution (2nm). The particular slit combinations employed for a given trace appear on the spectrum label. For example, the designation (22/11; 11/22) means that the excitation monochromator used to excite emission used 22 slits, and the emission was analyzed by scanning the emission monochromator using 11 slits. Conversely, the excitation spectra were recorded with the (scanning) excitation monochromator slits 11 and the emission monochromator slits 22.

Other Parameters--Phosphorescence occurs weakly if at all at room temperature. Since phosphorescence is the emission of greatest analytical importance for compounds studied here, all spectra were obtained at 77°K unless otherwise indicated.

The quartz sample tubes used for low temperature emission and analysis were optical quality, equivalent to Suprasil grade. The mean inside diameter of these tubes was 3 mm (with a variation of up to 15%), and wall thickness averaged about 0.5 mm. The tubes were typically 10 inches long and were closed (fused) at one end. In order to avoid possible transfer contamination, tubes were filled by pouring directly from the glass vial. With care and practice, this could be done without spillage. Tubes were usually filled to a depth of 7-8 cm, or a volume of about 0.5 ml. Sample tubes were closed with an improvised cap consisting of a short section of 1/8 x 1/4 inch rubber tubing stopped with a 3/16 inch diameter glass bead at one end. At room temperature, MCH diffuses through the rubber at a rate of about 0.05 ml per month so that samples should not be kept longer than this if initial concentrations are to be maintained.

After filling and capping the tube, it was lowered directly into liquid nitrogen in the optical dewar. About 30 seconds were required to completely immerse the sample into liquid nitrogen, at which time the sample was usually transparent and uncracked. Occasionally the sample may crack, even after repeated attempts to produce a glass; this is usually caused by residual moisture remaining in the tube after washing. Freshly washed tubes can be dried quickly by flushing several minutes with dry nitrogen gas.

Spectra of Pesticides--Spectra of the o,p'- and p,p'-derivatives of DDE, DDD, and DDT were discussed in the first report. It was found that the spectra of the DDD and DDT compounds are all very similar, and these compounds would be difficult to distinguish on the basis of luminescence spectra alone. The emission of DDE is very much weaker (about 400 times) than that of DDD or DDT, resulting in poor sensitivity. Spectra of p,p'-DDT were obtained in purified MCH at concentrations of 0.1, 1, 10, and 100 ppm. Solutions were prepared by successive ten-fold dilutions of a 100 ppm stock solution, prepared by dissolving 1 mg of DDT in 10 ml of solvent. All solutions were prepared and stored in screw-cap glass vials (4 dram) having both aluminum foil and Teflon cap liners.

A representative excitation/emission spectrum of p,p'-DDT is shown in Figure 4, and that of p,p'-DDT appears in Figure 5. As indicated above, both excitation and emission spectra are very similar, which is hardly surprising in view of the similarity in chemical structure (see Figure 1 of the first report).

At optimum resolution, the detection limit in MCH for DDT is about 0.03 ppm. Since DDD has a similar phosphorescence intensity, a similar detection limit is expected. This limit is largely imposed by the noise contributed by the light source and photomultiplier tube. Lower detection limits could of course be achieved by using wider analyzing slits, at the expense of resolution (and specificity).

A graph of phosphorescence intensity (approximated as the value of the signal maximum) versus concentration is shown in Figure 6. The analytical curve is linear except near 100 ppm, where departures become significant (about 25%).

Spectra of Aroclors--Monsanto Aroclors 1221, 1242, 1248, 1254, and 1260, along with several PCB isomers, were studied in the first report. Some of the more important highly chlorinated Aroclors (1248) and (1254) have been reinvestigated here, along with the newer aroclor 1016, a replacement for Aroclor 1242.

Spectra of Aroclors 1016, 1248, and 1254, in MCH appear in Figures 7-10. Solutions were prepared by successive ten-fold dilutions of a 100 ppm standard (1 mg per 10 ml solvent).

Because Aroclors are complex mixtures of PCB's, excitation/emission spectra obtained at different wavelengths should be somewhat different. This is indeed found to be the case, but much more so in the excitation spectra than in the emission spectra. Phosphorescence spectra of the more highly chlorinated Aroclors are broad, with maxima in the 440-470 nm region. An unexpected feature of the Aroclor 1016 phosphorescence is the double emission peaks near 450 and 470 nm (Figure 7). Comparison with excitation spectra of certain PCB isomers in the first report suggests that the emission components at short wavelengths (380-400 nm) are largely due to more highly chlorinated PCB isomers.

Spectral features of these Aroclors are largely independent of concentration below 10 ppm. At higher concentrations excitation intensities become distorted in the short wavelength region. An example of this is shown in Figures 9 and 10, which compare spectra of Aroclor 1254 at concentrations of 1 and 100 ppm (the 10 ppm spectrum is very similar to the 1 ppm). The attenuation at short excitation wavelengths in the more concentrated solution is due to strong absorption at these wavelengths. The excitation beam does not penetrate the sample, and the resultant emission occurs from a relatively shallow surface layer. Since the emission monochromator focuses more nearly at the center of the sample, this surface emission is not collected efficiently.

The theoretical dependence of emission intensity on concentration can be derived as follows. The emission intensity I_e is proportional to the intensity of the light absorbed by the sample I_a and the quantum yield of emission ϕ. The quantum yield is defined as the ratio of photons of light emitted to the number absorbed.

$$I_e = I_a \cdot \phi \tag{1}$$

Equation 1 neglects the possible existence of quenching mechanisms, such as collisional deactivation or energy transfer. This approximation is probably valid for most dilute solutions at low temperature. From Beer's Law,

$$I_a = I_o - I_o\, 10^{-\varepsilon bc} \tag{2}$$

where I_o is the intensity of the exciting light, ε is the molar extinction coefficient, b is the path length, c is the concentration. If the quantity (εbc) is suitably small (usually achieved by making c small), the exponential can be approximated as a series expansion giving

$$I_e \cong 2.303\, \phi\, I_o\, \varepsilon bc \tag{3}$$

Therefore the emission intensity is predicted to vary linearly with concentration at low concentration. Departures from linearity at high concentration imply that the series expansion applied to Beer's Law is no longer valid.

Phosphorescence intensities (approximated as peak heights) as a function of concentration are shown in Figure 6. Aroclor 1254 shows a linear response (within 20%) over the range 0.01 to about 60 ppm. Aroclors 1016 and 1248 were studied over a more limited range, but also appear to be linear below about 40 and 90 ppm respectively. The excitation wavelength employed for the Aroclor data of Figure 6 was 290 nm, which gives nearly optimum sensitivity for this Aroclor. The other Aroclors were excited at the same wavelengths as Aroclor 1254 for purposes of spectral comparison. However, the 290 nm excitation wavelength is not the optimum for Aroclor 1016 and 1248, whose excitation maxima are at shorter wavelength. In order to compare relative intensities more closely in Figure 6, intensity data for Aroclors 1016 and 1248 should be increased by about a factor of two.

The noise-limited detection sensitivity for Aroclor 1254 in MCH at 77°K is about 0.002 ppm at optimum resolution. On the basis of relative intensities, detection sensitivities for Aroclor 1016 and 1248 should be similar to that of Aroclor 1254.

Spectra of Aroclor/Pesticide Mixtures--A limited number of mixtures were studied in the first report, and these have been extended here. In particular, earlier work showed that Aroclor 1254 could be detected in the presence of DDT concentrations 100 times greater.

The mixtures studied here were composed of Aroclor 1254 (A) and p, p'-DDT (D) at concentrations ranging from 0.1 to 9 ppm in MCH:

a) 9 ppm A + 1 ppm D

b) 5 ppm A + 5 ppm D

c) 1 ppm A + 9 ppm D

d) 0.9 ppm A + 0.1 ppm D

e) 0.5 ppm A + 0.5 ppm D

f) 0.1 ppm A + 0.09 ppm D

Spectra of the above mixtures in MCH at 77°K appear in Figures 11-16. The spectral features of these mixtures are basically similar to the superposition of the spectra of the separate components. DDT is best measured free of Aroclor interference from an excitation spectrum monitoring emission near 380 nm. This is an emission wavelength where the DDT phosphorescence is appreciable (about 2/3 of its peak value), while the Aroclor 1254 emission is very low (about 2% of its peak value). Aroclor 1254 can be detected in the presence of DDT in two ways. First, the excitation spectrum in the 240-290 nm region could be obtained by monitoring emission at about 440 nm. Although DDT also has emission in this region, the Aroclor excitation spectrum begins at longer wavelength than that of DDT and thus can be measured free of DDT interference in this region. An alternate approach would be to excite at wavelengths of about 290 nm. The resultant emission would be principally that of Aroclor, depending on the excitation bandwidth employed; an excitation bandwidth of less than about 20 nm should give almost pure Aroclor 1254 emission.

In Aroclor 1016 and 1248, phosphorescence is also very weak at 380 nm, and this would remain a good wavelength to monitor the DDT excitation. However, the Aroclor 1016 or 1248 emission would not be excited free of DDT emission since the excitation spectra of these Aroclors are closer to that of DDT. In these cases, it would probably be preferable to excite Aroclor emission at about 285-290 nm with relatively narrow slits (less than 10 nm) and compensate for loss of intensity by using wider emission slits. Since the Aroclor emissions are intrinsically broad, bandwidths of up to 30 nm (10 nm for double-peaked Aroclor 1016) could probably be used without loss of spectral resolution.

Plots of the Aroclor 1254 emission intensity, based upon the peak height of the narrow excitation peak near 292 nm, show good linearity for concentrations between 0.1 and 10 ppm. DDT concentrations of this same magnitude thus appear not to affect linearity significantly.

Similar plots of the DDT emission intensity, based upon the narrow excitation peak at about 278 nm, show a similar linear dependence with concentration with one exception. The exception is the mixture having 1 ppm DDT and 9 ppm Aroclor 1254, and the intensity is about 50% lower than expected. The large departure from linearity may be due to energy transfer. PCB's have longer wavelength absorptions than DDT, which is equivalent to saying that their electronic states are at lower energies. The excited DDT molecules can transfer their energy to neighboring molecules, such as PCB's, having lower energy states (acceptors), and the probability of this varies approximately with the square of the acceptor concentration. The DDT emission is thus effectively quenched in the presence of high concentrations of Aroclor 1254. Energy transfer, however, does not appear to be important in the solution having 0.1 ppm DDT and 0.9 ppm Aroclor so that the absolute concentrations of the components seem even more important than their relative concentrations.

From the present study and the results of the first report, Aroclor 1254 could probably be determined in the presence of DDT concentrations of up to 100 times greater. DDT, having inherently weaker emission than Aroclor 1254, could probably be detected in the presence of about 20 times greater concentrations of Aroclor 1254. However, for absolute concentrations of Aroclor above about 10 ppm, there appears to be significant energy transfer from DDT to Aroclor. This is effectively a quenching mechanism, producing non-linearity in the analytical curve for DDT. This problem might be overcome by appropriate dilution of the mixture, provided that signal/noise considerations permit this.

Sources of Error--The uncertainty in a given intensity measurement averages about 25% and arises from several sources. First, quartz sample tubes vary by up to 15% in their diameters and are not perfectly straight. The latter condition results in non-uniform scattering of the excitation beam as the tube is rotated; the emission signal thus varies somewhat with tube rotation. This problem can be overcome by a rapid rotation of the sample tube during analysis so that inhomogeneities are averaged. A rotating sample cell of this type is described by Zweidinger and Winefordner (1970).

Another source of variation is related to the reproducibility and homogeneity of solute substitution in the frozen glass. This can be controlled somewhat by maintaining the same freezing rates, but variations of up to 10% have been found.

A potential source of error is contributed by the xenon source lamp. Using the water Raman band as a "standard," daily checks of the xenon lamp intensity at 350 nm showed random variations of up to 15% with the lamp intensity maximized. Of course slight defocusing of the arc so as to produce the same relative intensity would be possible. A better solution would be to ratio the sample emission to a signal proportional to the excitation light intensity; instrument gains should then be independent of lamp intensity fluctuations. This source of error can be minimized by running an appropriate luminescence standard (see Appendix).

SECTION VI

AROCLOR 1254 AND p,p'-DDT IN PURE WATER

These experiments were performed in order to determine recovery and extraction efficiency for Aroclor 1254 and p,p'-DDT in pure water.

Sources

Distilled water samples used originally were obtained from Belmont Springs Water Co., Belmont, Mass. This water comes in five-gallon plastic bags with a rubber hose outlet. Methylcyclohexane extracts of this water, when cooled to 77°K, showed a moderately strong but variable background emission. Based upon similarities in the UV absorption and phosphorescence excitation/emission of one of the principal impurities with that of dibutylphthalate, phthalates appear to be likely contaminants, with concentrations on the order of 10 ppb.

Although these contaminants could be removed by repeated solvent extraction, it was decided to try a different source of water. It was found that "Ultrapure" water obtained from Harleco contained negligible amounts of luminescent contaminants, and this material was used for subsequent experiments.

Originally, the solvent used for extraction was methycyclohexane, chosen largely because it would function both in this capacity and as the rigid solvent for low temperature luminescence measurements.

Use of this solvent for extraction was soon abandoned because the relatively high boiling point (101°C) required rather long concentration times. In addition, this solvent is fairly expensive at purities suitable for luminescence work.

The solvent selected for extraction, dichloromethane (DCM), has a low boiling point (42°C) and is obtainable in high purity at relatively low cost. Fisher Spectranalyzed DCM was used for all extractions.

One liter samples of Harleco water were doped with Aroclor or DDT directly into the original glass container. Dopant solutions were either 10 or 100 ppm solutions in ethanol, using the Aroclors and pesticides described in Section V. The ethanol was Graves Extra Fine, distilled prior to use to remove a phthalate-type contaminant. Glass lambda pipettes were used to deliver accurate microliter volumes of the dopant solution into water. The doped water was allowed to incubate overnight while being stirred continuously with a Teflon bar magnet activated by a magnetic stirrer.

Extraction Procedure

Extraction was performed in the same container. Seventy-five milliliters of DCM were transferred to the bottle and the mixture stirred on a magnetic stirrer for periods ranging from several hours to one day. The stirring speed was adjusted to be high enough to break the DCM into a fine emulsion which appeared to be uniform throughout the water.

After stirring, the bottle was removed from the magnetic stirrer and the DCM layer allowed to coalesce at the bottom of the bottle. This bottom layer, along with a few milliliters of water, was then transferred to a 125 ml separatory funnel using a 50 ml pipette. Approximately 20 ml of the original DCM added was retained by the water.

The DCM extract was dried using 10-20 grams of anhydrous Na_2SO_4. The Na_2SO_4 was Fisher Reagent grade material which had been previously heated to 400-500°C for several hours. Originally, the Na_2SO_4 was placed in a filter funnel (100 mm high, 23 mm I. D.) having a medium porosity glass frit. The DCM was then allowed to drip from the separatory funnel through the Na_2SO_4 layer into a small flask. This procedure usually required waiting several hours and was even longer with extracts of environmental samples containing particulates. Therefore this procedure was later modified by transferring the DCM to a 125 ml flask containing Na_2SO_4, adding a Teflon-covered bar magnet, and stirring the mixture on a magnetic stirrer for 15 minutes. Successive 75 ml extracts (two to three) were treated in the same manner and analyzed separately.

The use of DCM was suggested by the work of Hites and Biemann (1972). Various solvents and solvent mixtures have been used by other investigators, and these could probably be used in place of DCM. For example, Blumer (1970) has used n-pentane to extract organic compounds from sea water, Goerlitz and Brown (1972) recommend the use of n-hexane, and the EPA Method (1971) specifies a mixture of 15% ethyl ether in hexane. A disadvantage of DCM in standard procedures utilizing gas chromatography with an electron capture detector is the strong response of this type of detector to halogenated compounds.

Much higher detection sensitivities can be achieved by processing large volumes of water. The use of simple liquid-liquid extraction, however, would no longer be practical. Not only would large volumes of solvent be necessary, but even higher purity would be required to minimize interference from solvent impurities. For this reason, liquid extraction methods are replaced by flow systems employing adsorptive materials. The standard method of this type involves passing water through a column of activated carbon followed by Soxhlet extraction to remove adsorbed organics (Breidenbach et al, 1964).

More recently, Ahling and Jensen (1970) describe a filter consisting of a mixture of n-undecane and Carbowax 4000 monostearate on Chromosorb W to adsorb PCB's and chlorinated pesticides from water; the organochlorine compounds are eluted from the column with petroleum ether. Gesser et al (1971) and Uthe et al (1972) have reported the use of porous polyurethane foam plugs to absorb organochlorines, including PCB's from water. Harvey (1972) has reported the use of a crosslinked polymer resin (Rohm and Haas Amberlite XAD series) to adsorb PCB's and DDE/DDT compounds from marine waters. Although none of these methods was evaluated in this work, there appears to be no inherent difficulty in adapting these techniques to the present luminescence methodology. Of the above methods, that of Harvey (1972) would appear to be the least complex. It would be very worthwhile to determine how this procedure might best be coupled with low temperature luminescence analysis.

Concentration of the Extracts

Initially, extracts were concentrated using a Kuderna-Danish (K-D) concentrator, obtained from Ace Glass Company, Model 6707. This consisted of a 500 ml flask, a graduated 10 ml receiver, and a 3-ball Snyder column. The uppermost portion of the flask and the column were insulated with glass wool. The extract was put into the K-D, the receiver immersed in a water bath at 70-90°C, and the extract concentrated to approximately 1 ml (which required periods of several hours). Although a modification of this method might be desirable for batch processing of many samples, it was decided that the smaller number of samples analyzed here could be done more rapidly using a rotary evaporator.

The rotary evaporator used here was a Calab Model 5150 with Teflon fittings. During evaporations, the receiver flask was cooled with an ice water bath and the DCM distilled at room temperature under reduced pressure provided by a water aspirator. Typically, about 5 minutes were required to concentrate a 75 ml extract to less than one ml. A potential disadvantage of the rotary evaporator, in comparison with the Kuderna-Danish, is the problem of measuring small volumes of concentrated extract. No attempt was made to measure the final volumes of DCM, although as an estimate these were less than 0.5 ml.

Analysis of Extracts

Each concentrated DCM extract was first brought to a volume of 5-10 ml with methylcyclohexane. This solvent, unlike DCM, produces a rigid glass when cooled to 77°K. In addition, optical transparency is good throughout most of the ultraviolet. These volumes were originally chosen so that, if desired, absorbance measurements could be made easily in 1 cm cells, with enough excess to permit repeated measurements in the event of accidental sample loss or contamination. Sample volumes as small as 0.1 ml can be analyzed with the same equipment used here, although a more convenient sample size is approximately 0.5 ml.

Low temperature luminescence analysis was performed in the same manner as for the Aroclor/pesticide standards in MCH described in Section V.

Inclusion of about 10% DCM causes the MCH to freeze to a slightly cloudy glass. Intensities appear to average 2 to 3 times higher in this matrix than in pure MCH, probably because of increased optical path length due to scattering. Because of this scatter variability it is desirable to keep the final DCM volume as small as possible.

Recovery and Extraction Efficiency

Recovery of Aroclor 1254 from 1-liter samples of pure water was about 70% at the 1 ppb level and about 80% at the 10 ppb level. At both concentrations, 65-85% of the total amount extracted was contained in the first extract, and 95% or more in the first two extracts combined. Similar experiments with p,p'-DDT gave recoveries of about 50% at the 10 ppb level and about 70% at the 100 ppb level. Of the total extracted, 80-90% was contained in the first extract and 95% or more in the first two extracts combined.

The difference in recovery between Aroclor 1254 and p,p'-DDT is probably significant but is not presently understood. The observed losses are probably due mainly to adsorption on glass surfaces. Since the water samples were unfiltered, additional losses (presumably minor) due to adsorption on particulates may have occurred as well.

Detection Sensitivities

Detection limits for p,p'-DDT and for Aroclor 1254 in MCH at 77°K were found to be about 30 and 2 ppb respectively. If recoveries for p,p'-DDT and Aroclor 1254 are assumed to be 60% and 75% respectively, the detection limits for these compounds in a 1-liter volume of pure water would be about 0.5 and 0.03 ppb respectively. This assumes that the extraction solvent is free of potentially interfering contaminants.

SECTION VII

ENVIRONMENTAL SAMPLES

Grab samples were collected from several natural waters, mainly in the Boston area. The samples were taken within one foot of the surface. These were extracted and analyzed for PCB/DDT in a manner similar to that described for doped samples of pure water. River water samples were also doped with Aroclor 1254 and p,p'-DDT to determine recovery and sensitivity.

Sampling Locations

Samples were taken from the following locations:

	Location	Date(s)
A.	Shawsheen River, Bedford, Mass.	2/21/73
		5/24/73
		6/25/73
		9/26/73
B.	Concord River, Billerica, Mass.	8/23/73
C.	Hodgkins Cove, Gloucester, Mass.	10/2/73
D.	Milwaukee River, Milwaukee, Wis.	10/12/73
E.	Charles River, Cambridge, Mass.	12/6/73

The Shawsheen River is a shallow, narrow river close to the Baird-Atomic plant. This water does not appear to be seriously polluted, but is rich in natural organic material, giving it a yellow-brown appearance. Samples were collected where the river passes through two viaducts under the Middlesex Turnpike, a two-lane highway. The sampling bottle was held manually about three feet from the shore line (roughly half the width of the viaduct) and the surface water taken.

The Concord River, into which the Shawsheen flows, is also relatively unpolluted at the location sampled. In this case, a sample was taken near the approximate center of the river. This was done by lowering the sample bottle into the water from a bridge on Massachusetts Route 3. The bottle was contained in a bucket fashioned from an aluminum cylinder, secured by a rope.

The ocean water sample was taken near the University of Massachusetts Marine Station at Hodgkins Cove, Gloucester, Massachusetts. The point of collection was off a floating platform anchored about ten feet from shore. Several boats were anchored nearby, and streaks of oil were observed on the surface about six feet from where the sample was taken. The wind was calm, however, and no oil streaks were observed to enter the sample bottles.

Milwaukee River water samples were collected by A. Hornig on a return trip from that area. Other analyses of water in this area indicated PCB concentrations of several parts per billion (G. Veith, private communication). Samples were taken in Estabrook Park, on the east bank of the river, about 200 yards south of the northern boundary of the park. The samples were taken off the bank near the shoreline, where the water was less than a foot deep. The water did not appear to be heavily polluted, and no slicks, foams, or odors were noted. The water samples were yellow-green, as found for the Shawsheen and Concord River Samples.

The Charles River samples were collected near the center of the river by lowering bottles into the water from near the center of the Longfellow Bridge. This bridge carries moderately heavy car and subway traffic between Cambridge and Boston. Hites and Biemann (1972) performed extensive analyses of this river during 1971. Using combined gas chromatography-mass spectrometry and liquid chromatography, several pollutants including phthalates and polynuclear aromatics were identified.

Collection

Samples were collected in half gallon, wide-mouth glass bottles, with Teflon-lined plastic screw caps. Five milliliters of concentrated HCl, approximately 12 N., were added as soon as possible after collection to prevent possible biodegradation and to avoid extraction of basic compounds. Since organic pollutants may also be adsorbed on particulates, no attempts were made to remove these prior to extraction.

Extraction and Concentration

Sample bottles were returned to the laboratory and extracted with DCM in the manner described for pure water samples (Section VI). Some difficulties were encountered with these samples in that, after settling, the DCM extract consisted of a relatively clear lower layer, and an upper layer which appeared to consist of small globules suspended in a yellow-brown froth. This frothy mass was probably an emulsion of water in DCM, stablized by naturally occurring surfactants in the water.

Originally the DCM extract was dried by allowing it to drip through a short column of anyhdrous Na_2SO_4. The DCM passed through slowly until the frothy layer encountered the Na_2SO_4, whereupon the flow essentially stopped. This was probably because water in this layer hydrated the Na_2SO_4, forming a hard, largely impermeable crust. This procedure was later abandoned, and the DCM extract stirred with Na_2SO_4 in a small flask instead. After stirring, the extract was decanted off, concentrated, and diluted with MCH as described previously. Luminescence measurements, described in the following section, were performed on these solutions. The parameters used for each sample are summarized in Table 1.

Spectral Analysis

The natural water samples have a strong background luminescence, probably due to humic substances. In order to get representative signatures of this and potential organic pollutants, excitation and emission spectra were obtained at wavelengths other than those of analytical importance for PCB's or DDT. The wavelengths selected appeared suitable for excitation of the principal background components, as well as PCB/DDT. Wavelengths ordinarily used to excite emission were (in nm): 275, 290, 325, and 344. Wavelengths ordinarily used to monitor excitation spectra were: 380, 405, 430, and 465. Most spectra were obtained at 77°K using the SF-100 as described in Section V. Representative spectra are shown in Figures 17 through 29.

The various environmental samples were analyzed over a period of nearly ten months. During this time, both the xenon source lamp and the photomultiplier high voltage supply were changed several times. Also, the SF-100 collection mirror was cleaned occasionally. All of these modifications can influence the SF-100 gain setting so that these are not strictly comparable. However, spectra

TABLE 1

ANALYTICAL PARAMETERS USED FOR ENVIRONMENTAL SAMPLES

Collection Site	Date Collected	Water Volume in liters (no. bottles)	Volume HCl Added (ml) Per bottle	DCM Volume Per bottle, Per extract (ml)	DCM Stirring Time (hrs)	DCM Volume After Concentration (ml)	Figure No.
Shawsheen R.	2/21/73	1.7(1)	3	50	3	1	17, 19
Shawsheen R.	2/21/73	"	0	"	"	1	18
Shawsheen R.	5/24/73	"	5	75	11	1	20
Shawsheen R.	6/25/73	"	"	"	15	2*	21
Shawsheen R.	9/26/73	"	"	"	1	d**	22
Concord R.	8/23/73	"	"	"	96	1	23
Atlantic O.	10/2/73	6.8(4)	"	"	2	d**	24, 25
Milwaukee R.	10/12/73	3.4(2)	10	150	2	d**	26-28
Charles R.	12/6/73	1.7(1)	5	75	6	d**	29

*1 ml of DCM combined with 9 ml MCH.

**DCM extract evaporated nearly to dryness on rotary evaporator. Final volume not measured, but probably $\leq$ 0.5 ml. To this was added 10 ml MCH.

of the toluene impurity in MCH were taken routinely throughout this period to check the cleanliness of the quartz sample tubes. The relative intensity of this emission can be taken as rough "standard," although it has several shortcomings, such as the variation in toluene content between different bottles of the same solvent lot and irregularities in sample tube geometry. The intensities of the toluene emission indicate that the relative intensity as inferred from instrument gain settings are probably comparable within a factor of two, except for the first Shawsheen River sample (Figures 17 through 19), for which the instrument gain settings are about ten times higher.

Room Temperature Absorption Spectra--Room temperature absorption spectra were taken of a Shawsheen River extract (collected 2/21/73) in order to determine whether there was significant spectral information. In a solvent consisting primarily of MCH, spectra in the 240-460 nm region are very broad and bear some resemblance to those of humic and fulvic acids appearing in Schnitzer and Khan (1972); the spectra (in aqueous solution) shown in this work are diffuse throughout the region 200-400 nm with a broad maximum near 220 nm.

Although some PCB/DDT absorption could probably be discerned above this background at high concentrations, much greater sensitivity is obtainable from low temperature phosphorescence analysis with fewer potential interferences, since not all absorbing compounds emit. For these reasons, room temperature absorption measurements were discontinued.

Room Temperature Fluorescence--Since neither PCB's nor DDT-type compounds show significant fluorescence but do phosphoresce strongly at low temperature, room temperature analysis for these compounds is generally not profitable. However, some room temperature measurements were included simply to gauge the spectral behavior of background material. Also, in those instances where spectral evidence of PCB's (or DDT) is found, room temperature analyses may help confirm whether the observed emission is fluorescence or phosphorescence (without the need for a phosphoroscope).

As an example of the room temperature emission, an extract of Shawsheen River water in MCH is shown in Figure 19. The principal emission extends from about 300 to 600 nm, with an apparent maximum in the 400 nm region. The principal excitation spectrum is largely continuous from 220 to 400 nm, although several narrow bands appear above the continuum.

The continuous excitation spectrum is rather similar to that observed in the absorption spectrum and is again probably due to humic material. Shapiro (1957) has found that lake water contains 2-4 ppm of fluorescent yellow material. These compounds may be carboxylic acids, with a mean molecular weight of 456.

Fluorescence spectra obtained in this study are similar to those found for marine water. In marine water, the water soluble fluorescent substances are known as "Gelbstoffe" and are produced from decayed plant material. Representative spectra of Gelbstoffe appear in Figure 30, from Hornig and Eastwood (1973). Spectra in this report were obtained on untreated water samples at ambient temperatures in 1 cm rectangular curvettes. The principal excitation/emission peaks (uncorrected) are typically at 350 and 440 nm, obtained with the same instrument used for this work. These wavelengths differ somewhat for the river water extract, and these differences may arise from several sources including the biological history of the sample, possible fractionation of organic material by DCM, and spectral shifts induced by the solvent. Despite these differences, however, the origin of the background fluorescence in both fresh and marine water is probably due mostly to plant decomposition products.

Another natural component commonly observed in the water extracts is chlorophyll. An example of this emission appears in Figure 31. The emission consists of a single rather narrow band at about 670 nm, with an excitation maximum near 420 nm. This emission is fluorescence and would be observed at room temperature as well. The spectra of chlorophyll are well separated from DDT/PCB and would not constitute an interference.

Low Temperature Emission--The background features of the low temperature (77°K) spectra are generally similar to spectra obtained at room temperature, but with several important differences. First, the overall emission intensity is approximately three times greater at low temperature. This increase is too large to be attributed to volume shrinkage alone (about 20% for MCH) and probably reflects increased fluorescence yields due to diminished collisional and/or oxygen quenching.

In addition, much more fine structure appears above the broad background at low temperature. The most prominent structure consists of several sharp emission bands beginning at about 370 nm (Figure 17). The structure of this system bears a strong resemblance to that of pyrene (Figure 32), and the observed compound is probably a pyrene derivative. When this emission was first observed in Shawsheen River water, it was thought to possibly originate from auto exhaust condensate washed into the river from a nearby highway. Since then, similar structure has been found to some degree in all natural water samples and may suggest a natural origin. If this compound has a quantum yield similar to that of pyrene, its concentration in water is in the range 0.1 - 1 parts per trillion (ppt).

Another relatively sharp spectrum was observed in the Milwaukee River sample (Figures 26-28). The original MCH solution was deep yellow, and the high fluorescence intensity suggested that the concentrations were no longer in the linear region. For this reason the sample was diluted tenfold with MCH. Spectra of the diluted sample (Figures 27 and 28) had generally the same features as the undiluted, except for the appearance of a sharp band system originating at about 401 nanometers. Comparison of the excitation/emission spectrum with that of benzo(a)pyrene (Figure 33) suggests that this may be benzo(a)pyrene or a derivative. If this is benzo(a)pyrene, the concentration in water is on the order of 0.01 ppb. Since benzo(a)pyrene is a potent carcinogen, its possible presence in water is of interest. In a recent study of several New Hampshire rivers, Ellis (1972) has found trace (parts per trillion) concentrations of dibenz(ah)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, and fluoranthene. The fact that these compounds were found in relatively unpolluted waters led the author to suggest a natural origin.

River Water Doped with Aroclor 1254 and p,p'-DDT

These experiments were undertaken in order to establish recoveries and also to determine possible spectral changes induced in Aroclor 1254 by natural organic matter; for example, by differential adsorption or extraction of PCB isomers. In both cases solutions were doped with ethanol solutions of Aroclor 1254 and p,p'-DDT as described in Section VI.

Solutions were mixed thoroughly on the magnetic stirrer for periods ranging from one hour to one day. Extraction was done by addition of successive 75 ml aliquots of DCM. Stirring with DCM was done on the magnetic stirrer, with the solution agitated sufficiently to break the DCM into a fine mist throughout the water; extraction times were one hour or longer. The DCM extracts were dried, concentrated, and diluted with MCH in the manner described previously for undoped samples.

Spectral Features--Figures 34 and 35 show spectra obtained for the first extract of 6 and 60 ppb Aroclor 1254 in 1.7 liter samples of Shawsheen River water. These samples were stirred continuously for one day after doping, then extracted three times with 75 ml portions of DCM (each extract stirred for one hour).

Both samples were stirred continuously for one day after doping, and then extracted three times with DCM (each extract stirred one hour).

The Aroclor 1254 emission maximum at 440 nm at the 6 ppb level is largely lost in the background; excitation spectra monitored at 430 and 465 nm do show the expected Aroclor peaks near 260 and 290 nm. Spectra of the 60 ppb solution show distinct Aroclor excitation and emission signatures. The structure of both excitation and emission does not appear to be significantly different from that of the Aroclor 1254 standard in MCH (Figure 9).

A similar experiment was done by spiking p,p'-DDT into another sample of the same water at concentration of 60 ppb. Whereas the emission spectrum, peaked at about 400 nm, is largely obscured by the background, the sharp excitation peaks at about 240 and 278 nm are clearly visible (Figure 36).

Recovery and Extraction Efficiency--Recovery of Aroclor 1254 from the natural water samples was 19% for the 6 ppb solution and 27% for the 60 ppb solution. Of the amount extracted, 75-80% was contained in the first extract and an additional 15% or more in the second extract (both concentrations). A third extract of the 60 ppb sample contained about 1% of the total.

Recovery for a similar water sample spiked with p,p'-DDT to a concentration of 60 ppb was about 35%. Of the amount extracted, about 80% was contained in the first extract and 15% in the second.

Reducing the incubation period with increased extraction time did not substantially improve recovery. For example, a 6 ppb sample of Aroclor 1254 in Shawsheen River water incubated for one hour and stirred with DCM for 15 hours still gave only a 24% recovery. Recoveries from river water are thus much lower (2-4 times) than from pure water. Possible reasons for this are discussed in a later section.

Detection Sensitivities--The detection limit for Aroclor 1254 spiked into raw river water of the type used here is about 2 ppb, and for p,p'-DDT is about 10 ppb. These rather high values are due to low recoveries and to the presence of large amounts (probably several ppm) of luminescent background material in natural water. Removal of this background material prior to analysis should result in much lower detection limits. Assuming a recovery of about 25%, the detection limits of Aroclor 1254 and p,p'-DDT should be on the order of 0.1 and 1 ppb respectively for sample volumes of about one liter. Larger water volumes (and higher recoveries) would of course permit better sensitivity.

Discussion--The low recovery obtained for the environmental samples may be due to adsorption by particulates. Golden and Sawicki (1973) have investigated ultrasonic extraction for removal of aromatic hydrocarbons from air particulate matter collected on glass fiber filter paper. In order to quickly establish the potential of this technique for the problem at hand, the particulates from the 60 ppb Aroclor sample were placed in a small flask along with about 10 grams of Na_2SO_4 and 100 ml of DCM. The mixture was then subjected to 50 watts of ultrasonic power from a 1/4" diameter probe for about ten minutes. The probe was passed through the slurry to promote better contact. Subsequent concentration and analysis of the DCM revealed only a very small (about 1% of the previous total) additional quantity of Aroclor 1254 had been removed. However, the fact that some improvement was obtained suggests that this technique deserves further study. For example, higher power levels and improved contact between probe and solids might give better recovery.

Aside from adsorption by particulates, low recovery may be a consequence of enhanced water solubility of these compounds by humic substances. Wershaw et al (1969) have found that a 0.5% sodium humate solution in water solubilizes DDT. The DDT solubility in pure water is normally about 40 ppb (Babers, 1955) but is at least twenty times larger with sodium humate. The sodium humate may function as a sulfactant which stablizes a dispersion of DDT in water. Since filtration of DDT-humate solutions through a 0.45μ membrane filter did not appreciably affect DDT concentration, emulsion particles would have to be smaller than this.

Still another possibility is that the low recoveries are only apparent, being a consequence of the measurement technique. In particular, natural compounds might quench the DDT or PCB phosphorescence by serving as energy transfer acceptors. That is, rather than phosphorescence, the organochlorine may transfer its excitation energy (light absorbed) to other molecules nearby which subsequently emit. This mechanism does not appear to be strongly operative for p,p'-DDT or Aroclor 1254. This was demonstrated by doping MCH extracts of river water with solutions of Aroclor and DDT in MCH. In both cases, the intensities were found to be the same as obtained in MCH alone within experimental error.

Evidence of PCB/DDT in Natural Waters

None of the environmental samples showed clear evidence of DDT-type compounds. The only environmental sample showing evidence of PCB's was the marine sample taken near Gloucester, Mass. Extracts of this sample show excitation peaks above the background continuum in the 280-290 nm region. Of the Aroclors studied, the observed excitation peaks most closely resemble Aroclor 1254, although even here the resemblance is not strong; this Aroclor should have a moderately strong excitation band in the 250-260 nm region which is not apparent in the environmental sample. If the observed s structure is assumed to be that of Aroclor 1254, its concentration in water would be about 1 ppb. This value assumes 100% recovery, although experience with spiked samples suggests that actual recoveries are probably 20-30%. Harvey et al (1972) have found levels of 1-150 ppt (determined as Aroclor 1254) in the open North Atlantic, with higher concentrations occurring nearer the surface. Higher concentrations, as may be present for the sample collected here, are expected in coastal areas.

Excitation spectra of the marine sample obtained at several emission wavelengths show that there are two separate excitation peaks in the 280-290 nm region. Monitoring emission at 400-440 nm emphasizes a peak at 284 nm, while monitoring at longer wavelengths emphasizes a peak near 290 nm. The later peak is at the wavelength characteristic of Aroclor 1254, whereas the former is more characteristic of a phthalate, perhaps di-2-ethylhexyl phthalate (DEHP) or the closely related diisodecyl phthalate (DIP). Spectra of dibutyl phthalate (DBP) are discussed in more detail in the following section.

The excitation peak at about 290 nm may in fact be due to a phthalate, since the secondary absorption of Aroclor 1254 in the 250-260 nm region is not evident. If one or both of these bands are due to phthalates, their concentration in water would be on the order of 1-10 ppb, assuming extraction efficiencies of 100%. Hites (1973) and Hites and Biemann (1972) report finding phthalates in the Charles River (Boston) at levels of 1-2 ppb. Corcoran (1973) has reported finding about 0.6 ppm phthalate, probably DEHP, in the lower Mississippi River.

Background and Interferences

Humic Substances--As discussed earlier, the principal emission of natural organic matter present in water consists of a relatively broad background in the 300-600 nm region for excitation wavelengths below 300 nm. The maximum of this background emission is in the 400-450 nm region, which is also the location of the phosphorescence of DDT-type compounds and the more highly chlorinated Aroclors. Since the emission of these compounds is also broad, they are not easily distinguished from background at low concentrations. The interference is less severe, however, if excitation spectra are monitored. This is because the excitation spectrum of the background material is usually continuous in the 220-300 nm region where the absorption of DDT and PCB's occur. The excitation spectra of the latter compounds, particularly DDT, are relatively narrow and can therefore be more easily distinguished above the background continuum.

It would be desirable for future work to first remove as much of these natural organics as possible, particularly those emitting in the 400 nm region. Several simple approaches seem attractive. First, if the majority of the fluorescent compounds are acids, as suggested by Shapiro (1975), making the water sample basic should convert the acids to the much more soluble salts. These salts should have much lower solubility in DCM than the acids and thus remain primarily in the aqueous phase. To test this, two identical 1.7 liter samples of Shawsheen River water were extracted with DCM and the concentrated extracts diluted with MCH and analyzed in the usual manner. One water sample had been treated with 5 ml concentrated HCl, and the other with

2.5 grams NaOH. Unfortunately, the alkaline sample showed only a very slight (about 10%) reduction in background intensity. The quantity of NaOH used here was chosen to provide a hydroxide ion molarity equivalent to that of the acid solution and may not have been adequate. This experiment should probably be repeated at even higher pH values.

Blumer (1970) has used a simple chromatographic operation on silica gel to separate components of marine water into three groups: saturated hydrocarbons, olefinic/aromatic compounds, and polar materials. For luminescence analysis, it would probably suffice to remove only the polar substances from the remaining compounds since saturated compounds would have negligible luminescence. In Blumer's procedure, pentane is used as the extractant and is also used to elute saturated, olefinic, and aromatic compounds from the chromatographic column.

A similar procedure has been recommended by Johnson (1971). In this approach, components are eluted from a silica gel column using benzene-hexane mixtures. Aromatic chlorinated pesticides are thus separated from chlorinated aliphatics and from various polar compounds. The possible disadvantage of this technique for luminescence analysis is the presence of benzene, which is itself luminescent. However, the procedure of Blumer discussed above suggests that hexane alone might be used to elute all except polar materials.

To summarize, luminescent natural substances (probably largely polar) should be removed prior to analysis. This may be possible by proper adjustment of the pH of the water preceding extraction. If this proves unsuccessful, the silica gel cleanup procedure of the type used by Blumer should be contemplated.

Phthalates--An important class of compounds which can interfere with PCB determination are phthalates (phthalic acid esters). These compounds, particularly di-2-ethylhexyl phthalate (DEHP) are commonly used as plasticizers for polyvinyl chloride. These compounds may be even more prevalent in the environment than PCB's. Total domestic sales of Monsanto PCB's peaked at about 75 million pounds in 1970 but have declined steadily since then (Nisbet and Sarofim, 1972). Sales of phthalates, however, reached nearly a billion pounds in 1970 and have probably risen since then (Graham, 1973). The water solubility of these compounds may be greater than that of PCB's. Wallnofer et al (1973) give water solubilities of many PCB isomers at 22-24°C as being in the range 0.001 to 6 mg/100 ml; higher chlorination generally gives reduced solubility. In contrast, the solubility of dibutylphthalate is 40 mg/100 ml (Handbook of Chemistry and Physics).

Phosphorescence excitation/emission spectra of diisodecyl phthalate (DIP) and dibutyl phthalate (DBP) are shown in Figures 37 and 38. The spectrum of di-2-ethylhexyl phthalate (DEHP), being chemically very similar to DIP, should strongly resemble that of DIP. The emission spectra are similar in structure and wavelength to those of the more highly chlorinated Aroclors. The excitation spectra resemble that of Aroclor 1254 in the long wavelength region but lack strong secondary absorptions in the 250-260 nm region. The phosphorescence intensities of DIP and DBP in MCH (77°K) are roughly factors of 7 and 2 less than the same weight of Aroclor 1254.

If only DIP or DBP (or some mixture of these) were present, excitation structure monitored at several emission wavelengths should be nearly the same. In the case of an Aroclor, which consists of many isomers, rather different excitation spectra are obtained depending on the emission wavelength chosen.

Another parameter which should provide some discrimination between phthalates and highly chlorinated PCB's is the phosphorescence lifetime. The mean lifetime of Aroclor 1254 should be similar to that of an "average" biphenyl isomer having five chlorine atoms. Dreeskamp et al (1972) have measured the phosphorescence lifetimes of several chlorinated biphenyl isomers. Lifetimes given for 2,2', 4,4'-tetrachlorobiphenyl and 2,2', 4,4', 6,6'-hexachlorobiphenyl are 0.11 and 0.05 seconds respectively in EPA glass at 77°K. (EPA is a mixture of ethanol, isopentane, and ether in the proportions 2:5:5 by volume.) Dubinskii (1959) has determined the lifetime of DBP as 0.80 seconds in ethanol at 93°K; the lifetime of DIP is probably similar. Thus the common phthalates probably have phosphorescence lifetimes up to ten times longer than the more highly chlorinated PCB's. This lifetime difference can be utilized as follows: Suppose one has an arbitrary mixture of Aroclor 1254 and DBP. If one allows a time delay of 0.80 seconds between excitation and detection, the intensity of the DBP emission will have fallen to 0.37 (1/e) of its original value, whereas the intensity of the Aroclor 1254 emission, assuming a mean lifetime of 0.08 seconds, will have dropped by a factor of 5 x 10^{-4}. Thus, by using time discrimination, phthalates could probably be determined in the presence of much higher concentrations of highly chlorinated PCB's. Time discrimination of this type can be achieved using a phosphoroscope, which is simply a mechanical light chopper which introduces a time delay between excitation and detection. A drawback with devices of this type is that usually much intensity is lost. Alternatively, the source can be pulsed, but this is no longer in the realm of a simple laboratory experiment.

To summarize, certain phthalates may interfere with the more highly chlorinated PCB isomers. Although intrinsic differences in phosphorescence lifetimes should permit some discrimination, this would require more sophisticated instrumentation than employed in the present study.

Accuracy and Precision

In order to estimate accuracy and precision, separate estimates of these are needed for both the extraction and luminescence procedure. The luminescence measurement itself is probably reproducible to about 10% for a given sample frozen repeatedly to 77°K; this variability arises in the differences of sample cooling rate. This further assumes that normal variations in the xenon arc intensity are properly compensated by use of a standard. If the same sample is analyzed in different quartz tubes, additional variations of up to 15% can be expected due to the variability in quartz tube dimensions. The accuracy of a given determination, using a randomly selected quartz tube, would probably be better than 25% presuming that the analyte concentration were not too high (i.e., beyond the range of linearity).

The error introduced by the extraction procedure is less easily established since recoveries of the compounds of interest are probably dependent on both the nature of the container (e.g. adsorption on glass) and the presence of other materials in water (e.g., particulates and natural surfactants). Also, determination of recoveries by the luminescence method are also subject to the inaccuracies of the latter as discussed earlier. An intensive study of this subject was not undertaken, but most determinations of PCB/DDT doped into water were run in duplicate, with an estimated precision of about 15%.

Recoveries from natural waters were as low as 20%, so that if typical recoveries were completely unknown, concentrations could be too low by up to a factor of five. If recoveries have been determined for a particular type of sample, the accuracy should be roughly equal to the estimated precision (15%).

For the combined extraction/luminescence analysis, the accuracy and precision are approximately the sum of the values for extraction and luminescence determination separately. These estimates have been summarized in Table 2.

TABLE 2

ESTIMATED ACCURACY AND PRECISION OF
THE LUMINESCENCE METHOD

	Accuracy (%)	Precision (%)
Luminescence Measurement*	10 - 25	~10
Total Analysis** (Extraction + Luminescence)	25 - 40	~25

*Estimates based on measurement of standard solutions in methylcyclohexane at 77°K using a randomly selected sample tube.

**Estimates based on recoveries of Aroclor 1254 and p,p'-DDT from doped water samples.

Improved estimates of accuracy and precision require that recoveries be separately determined for the particular type of water sample under investigation. Since the estimates given here are subject to errors in the luminescence measurement, recoveries should preferably be checked using an independent method such as gas chromatography.

SECTION VIII

SUMMARY

Basic studies consisted of the documentation of the low temperature luminescence spectra of several PCB mixtures (Aroclors) and DDT-type compounds, and the application of these results to the development of an analytical procedure for these compounds in water. A detailed description of the instrumentation and methodology is given in the Appendix.

Spectra of Aroclors, Pesticides, and their Mixtures

Luminescence spectra of several PCB isomers, Aroclors, and DDT derivatives were given in the first final report of the present program (Brownrigg et al 1972). Since some of these data were obtained on a slightly different instrument than used for the present work, spectra of some of the more important Aroclors and pesticides have been repeated here. Compounds studied were p,p'-DDD and DDT, and Aroclors 1016, 1248, and 1254.

Luminescence measurements were performed at liquid nitrogen temperature (77°K) using a standard Baird-Atomic SF-100 Fluorispec. The solvent selected was methylcyclohexane, which forms a clear glass when frozen rapidly to 77°K. Trace amounts of luminescence impurities (mainly toluene) present in the solvent were removed by column chromatography using silica gel. Removal of these impurities resulted in improved detection sensitivites over those given in the first report.

The phosphorescence intensities of p,p'-DDT and the three Aroclors were found to be linear with concentration below about 10 ppm. Detection limits for DDD/DDT and the Aroclors are about 0.03 and 0.002 ppm respectively, using highest spectral resolution (2nm). Limits are imposed primarily by source/phototube noise, and could be improved by using wider slits at the expense of resolution.

Several mixtures of Aroclor 1254 and p,p'-DDT were studied with concentrations ranging from 0.1 to 9 ppm. The phosphorescence intensity of each component was linear over the concentration range, except for the solution containing 1 ppm DDT and 9 ppm Aroclor 1254, in which the DDT intensity was almost 50% lower than expected. The observed departure from linearity is thought to be caused by energy transfer quenching from DDT to PCB molecules. Since a ten-fold dilution of this solution behaved normally, a possible solution to problems of this type might be sample dilution, provided loss of sensitivity were not too large.

Aroclor 1254 and DDT in Pure Water

Distilled water samples of approximately one liter were doped with Aroclor 1254 and p,p'-DDT (in ethanol solution) in order to determine recoveries and extraction efficiencies.

After allowing the samples to stir for up to a day, samples were extracted with two or three successive 75 ml volumes of dichloromethane (DCM). This particular solvent was selected for extraction because of its low boiling point, low cost, and generally high purity. Extraction was accomplished by stirring the mixture vigorously for at least one hour with a Teflon-coated bar magnet, activated by a magnetic stirrer. Extracts were dried with 10-20 grams anhydrous sodium sulfate and concentrated almost to dryness with a rotary evaporator. To the concentrated extract was then added 5 or 10 ml of MCH, and the luminescence analyzed at 77°K.

Recovery of Aroclor 1254 was about 70% at the 1 ppb level and about 80% at the 10 ppb level. Of the total amount recovered, 75-85% was contained in the first extract and 95% of more in the first two extracts combined. Similar experiments with p,p'-DDT gave recoveries of about 50% at the 10 ppb level and 70% at the 100 ppb level. Extraction efficiencies were similar to that for the Aroclor, with 80-90% of the total in the first extract and 95% or more in two combined extracts.

The difference in recovery between Aroclor 1254 and DDT is probably significant but is not presently understood. Observed losses are probably due mainly to adsorption on glass surfaces. In addition, since the water samples were not filtered, some additional (presumably minor) losses due to adsorption on particulates may have occurred.

Detection limits for DDT and Aroclor 1254 in one-liter samples of pure water are estimated at 0.5 and 0.03 ppb, assuming recoveries of 60 and 75% respectively.

Aroclor 1254 and DDT Doped into Natural Water

Samples of water (1.7 liters) taken from a nearby river were doped with Aroclor 1254 and p,p'-DDT and analyzed in the same manner as described for the pure water samples. The river water samples were yellow-green and contained particulates, but were left unfiltered for these studies.

Recoveries obtained for Aroclor 1254 were 19% at the 6 ppb level and 27% at the 60 ppb level. Of the total extracted, 75-80% was in the first extract and an additional 15% or more in the second. Recovery for a similar sample doped with p,p'-DDT at the 60 ppb level was about 35%, with 80% of the total in the first extract and 15% in the second.

The recoveries from raw river water are much lower (2 to 4 times) than from pure water. This is thought to be due primarily to increased adsorption by particulates, but increased solubilization by humic material may also be responsible. Possible quenching of PCB/DDT by other substances present in water does not appear to be significant. It would have been of interest to determine the relative importance of these mechanisms by analyzing filtered river water. However, since a proper definition of natural water should probably include suspended particulates, no attempts were made to remove these from any of the environmental water samples prior to analysis.

Detection limits for Aroclor 1254 and p,p'-DDT in 1.7 liter volumes of raw river water used here are about 2 and 10 ppb respectively. These high values are due to low recoveries and the presence of a strong background luminescence from natural substances, probably humic material. If these compounds (presumably polar aromatics) were removed prior to analysis, detection limits for Aroclor 1254 and DDT could probably be reduced to approximately 0.1 and 1 ppb for a one-liter sample volume, assuming 25% recovery. Improved extraction techniques and analysis of larger sample volumes could further reduce these limits.

Environmental Water Samples

Grab samples of 1.7 liters were taken from three rivers in the Boston area and also from the ocean near Gloucester, Massachusetts. A sample of water from the Milwaukee River was collected by A. Hornig on a return trip from this area. All samples were acidified with 5 ml concentrated HCl and then extracted and analyzed in the same fashion as described for the pure water samples.

All samples show broad background emission peaking in the 400 nm region which is probably due to humic material, particularly aromatic carboxylic acids and phenols. Narrow bands are observed superimposed on the broad background emission. One particular system, probably due to a pyrene derivative, is observed in all water samples. If this material were pyrene, its concentration in natural water would be in the range of 0.1 - 1 ppt. The wide distribution of this compound suggests a natural origin. Another sharp spectral system was observed in a sample of Milwaukee River water. This spectrum resembles that of benzo(a)pyrene or a derivative; it this is a correct identification, the concentration in water would be on the order of 10 ppb.

The broad emission contributed by humic substances overlaps the similarly broad emission of the Aroclors and DDT and therefore constitutes an interference. However, the excitation spectra of the humic materials appears to be nearly continuous in the 220-300 nm region where the excitation (absorption) spectra of PCB's and DDT occur. Since the excitation spectra of the latter compounds, particularly DDT derivatives, are much narrower, they are more easily distinguished above background than are the emission spectra. Even so, the background emission contributed by humic materials diminishes sensitivity and future studies should be directed at removing these prior to analysis.

None of the environmental samples studied shows evidence of DDT-type excitation peaks, implying DDT concentrations of less than 10 ppb. Only one of the samples, namely a sample of marine water taken near Gloucester, Massachusetts, shows evidence of excitation peaks in the region expected for Aroclors. Two distinct excitation peaks are observed at approximately 284 and 290 nm. The latter is close to the value expected for Aroclor 1254, although a secondary Aroclor peak near 260 nm is not evident. The 284 peak resembles that obtained for diisodecyl phthalate, and both this peak and the one near 290 nm may in fact be due to phthalates. If this is the case, the phthalate concentrations found would be on the order of 1-10 ppb. In general, the spectra of phthalates are similar enough to those of Aroclors to result in possible interference, and further work on this problem is necessary.

Methodology

The results of the experimental work performed in this study have been analyzed, and a first cut has been taken in producing a practical "method" which appears as an appendix. The method considers extraction techniques to prepare a water sample for instrumental analysis, detailed instructions for specific instrument settings to be used, and data interpretation.

Grab samples of water are collected, extracted with dichloromethane, and the combined extracts dried and concentrated. Methylcyclohexane is then added to the concentrated extract and luminescence analysis performed at liquid nitrogen temperature (77°K). Identification and estimation of Aroclors and DDT derivatives are based upon comparison of the unknown excitation/emission signatures and intensities with those of appropriate standards in methylcyclohexane.

Known samples of Aroclors and DDT in methylcyclohexane are used to calibrate the fluorescence instrument. Doped water samples are used to gain familiarity with the extraction and concentration techniques, and environmental samples are treated in a similar fashion.

Recommended wavelengths, slit widths, and other operational parameters for the fluorescence instrument are given. Aroclor/DDT spectra shown in the main text can be used as a guide. Apparatus and chemicals necessary for collection and extraction of water samples are described. Finally, accuracy, detection sensitivities, and possible interferences are discussed.

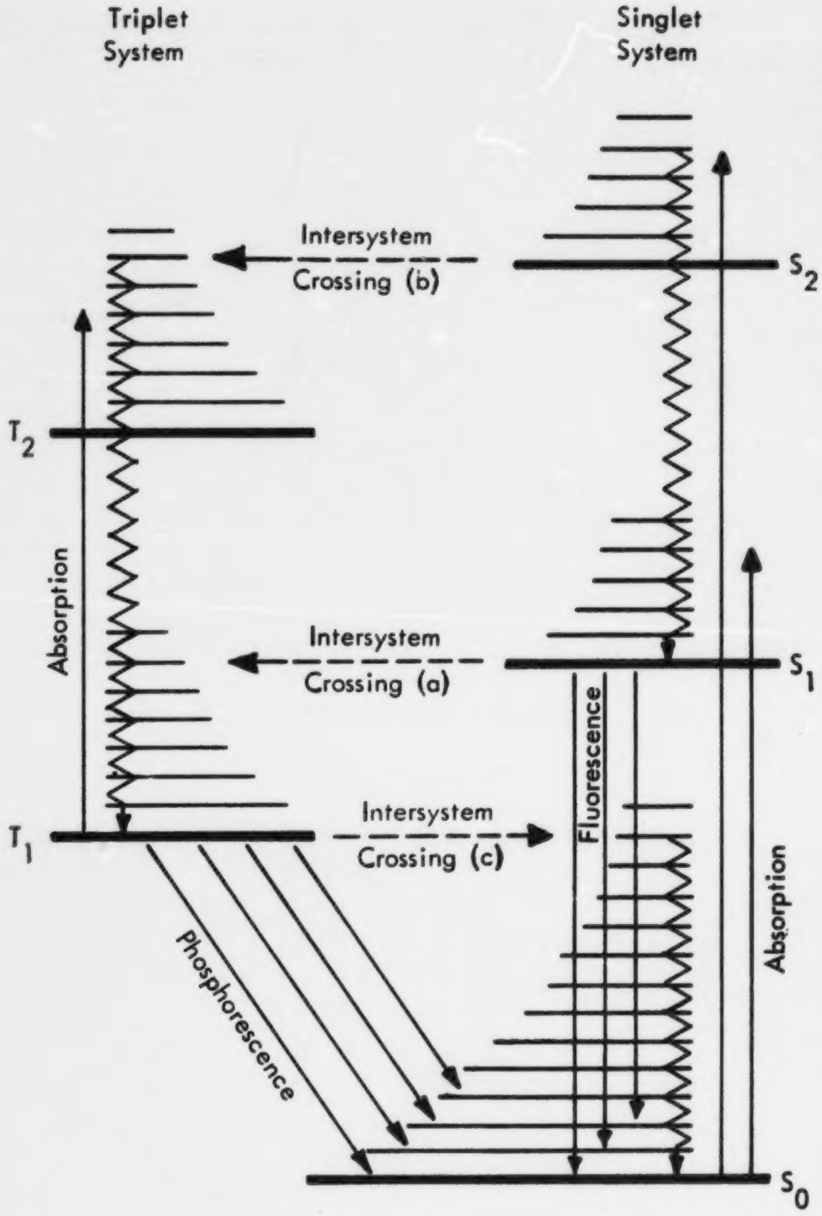

Triplet System
Singlet System
Intersystem Crossing (b)
S2
T2
Absorption
Intersystem Crossing (a)
S1
Fluorescence
Intersystem Crossing (c)
T1
Phosphorescence
Absorption
S0

FIGURE 1. SCHEMATIC DIAGRAM OF MOLECULAR TRIPLET AND SINGLET ENERGY LEVELS

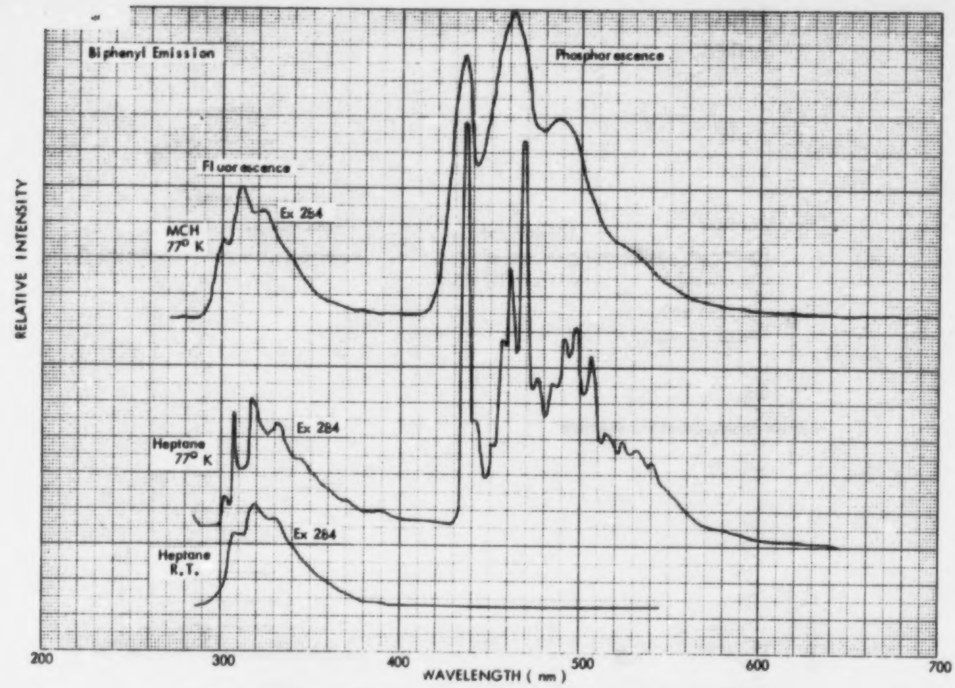


FIGURE 2. BIPHENYL EMISSION IN METHYLCYCLOHEXANE (MCH) AND IN HEPTANE

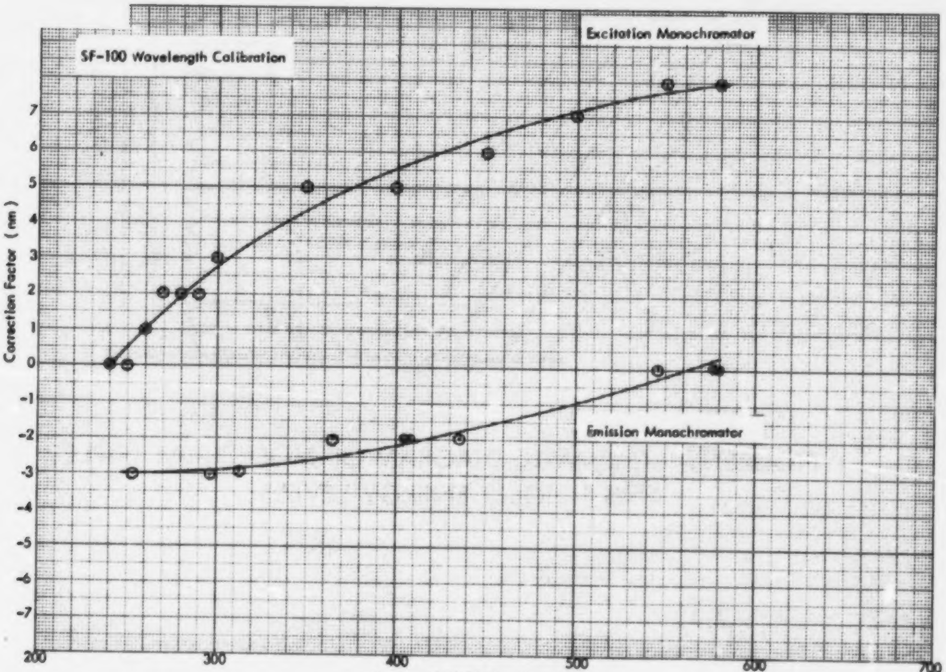


FIGURE 3. WAVELENGTH CALIBRATION CURVES FOR THE SF-100 FLUORISPEC USED IN THIS STUDY

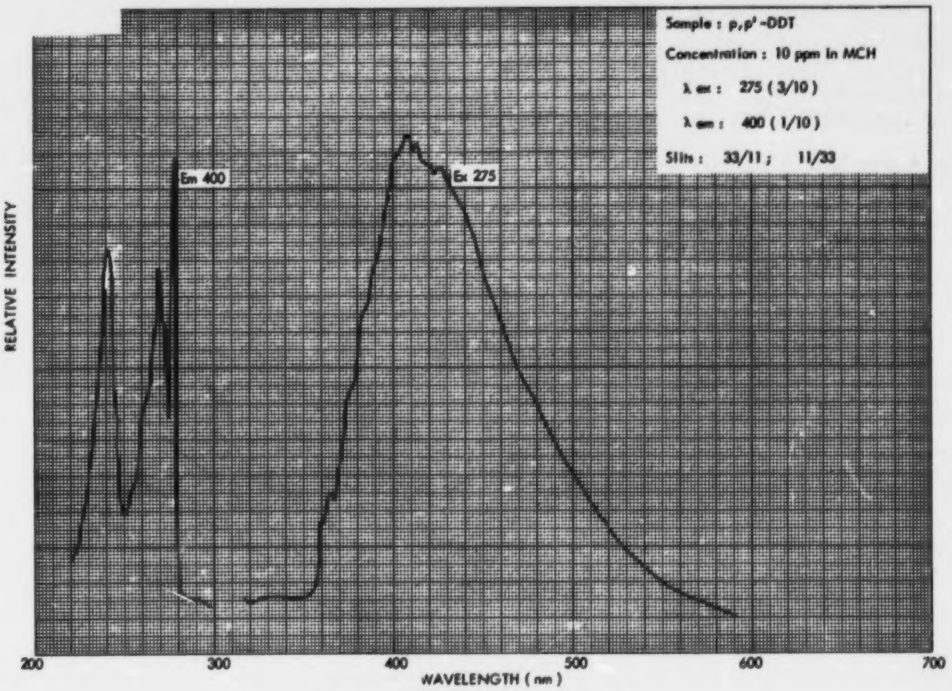


FIGURE 4. P, P'-DDT, 10 PPM IN MCH, 77°K

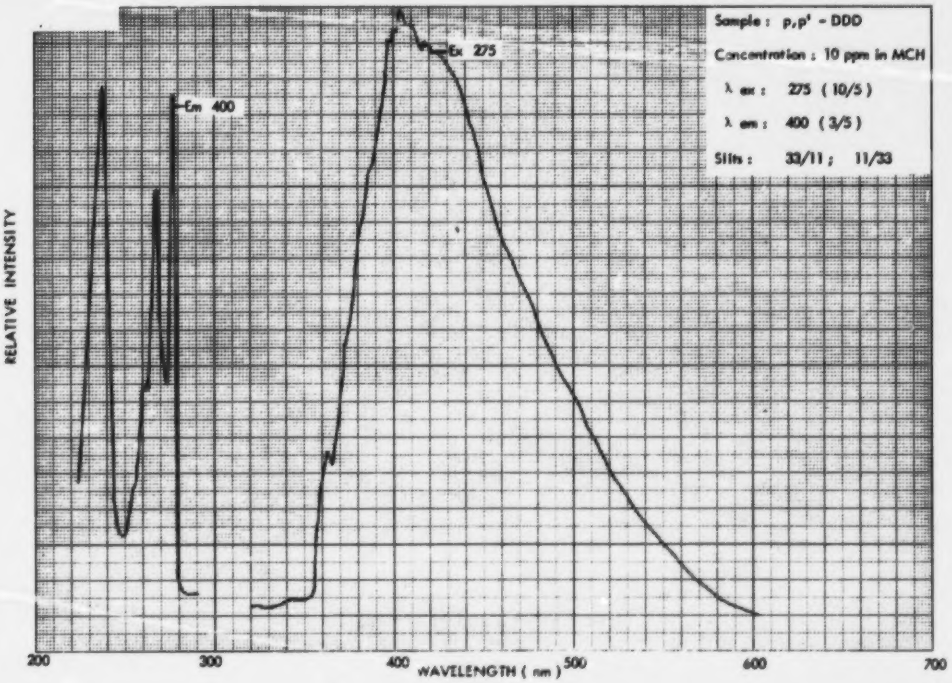


FIGURE 5. P, P'-DDD, 10 PPM IN MCH, 77°K

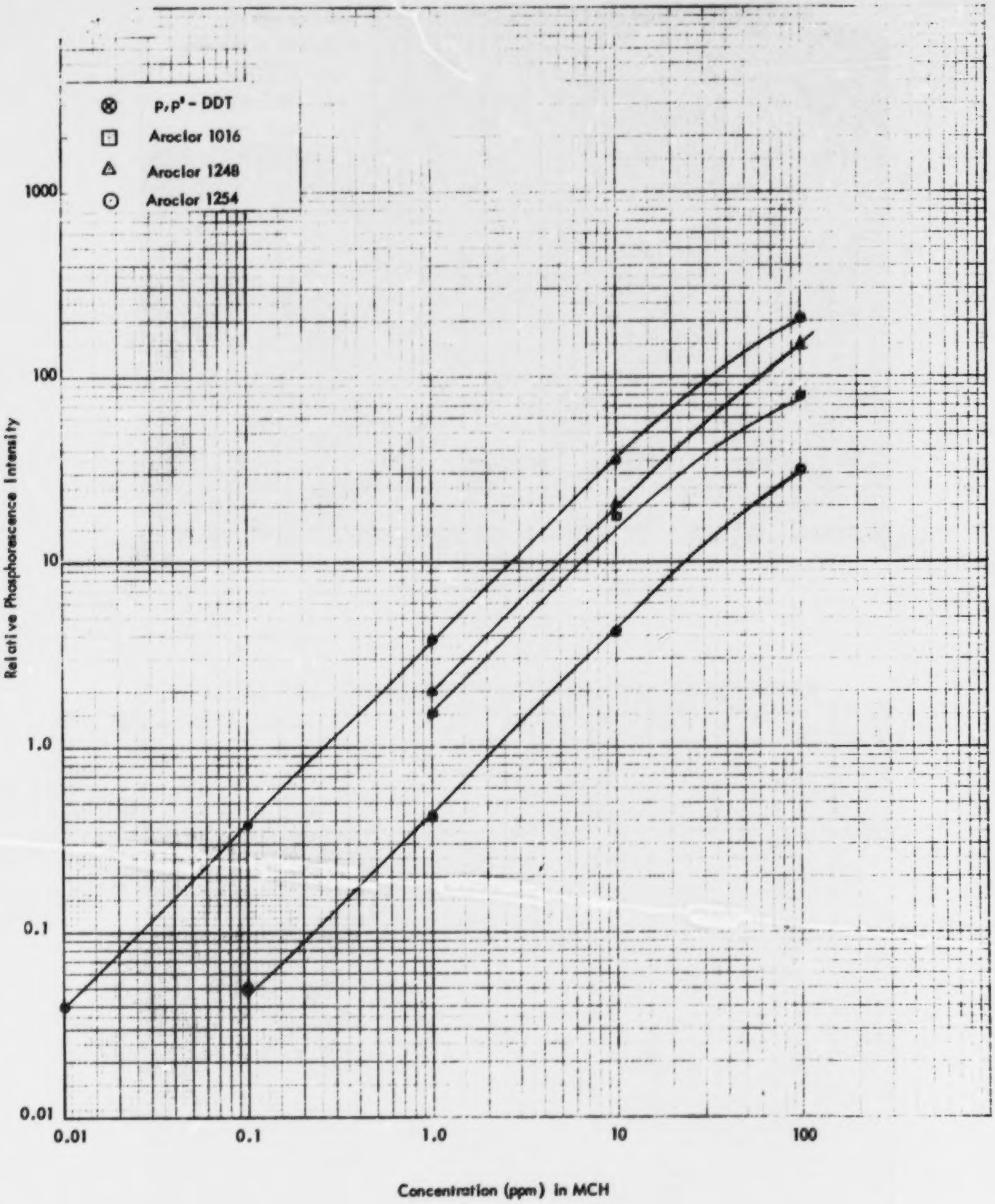


FIGURE 6. ANALYTICAL CURVES FOR P, P'-DDT AND SEVERAL AROCLORS

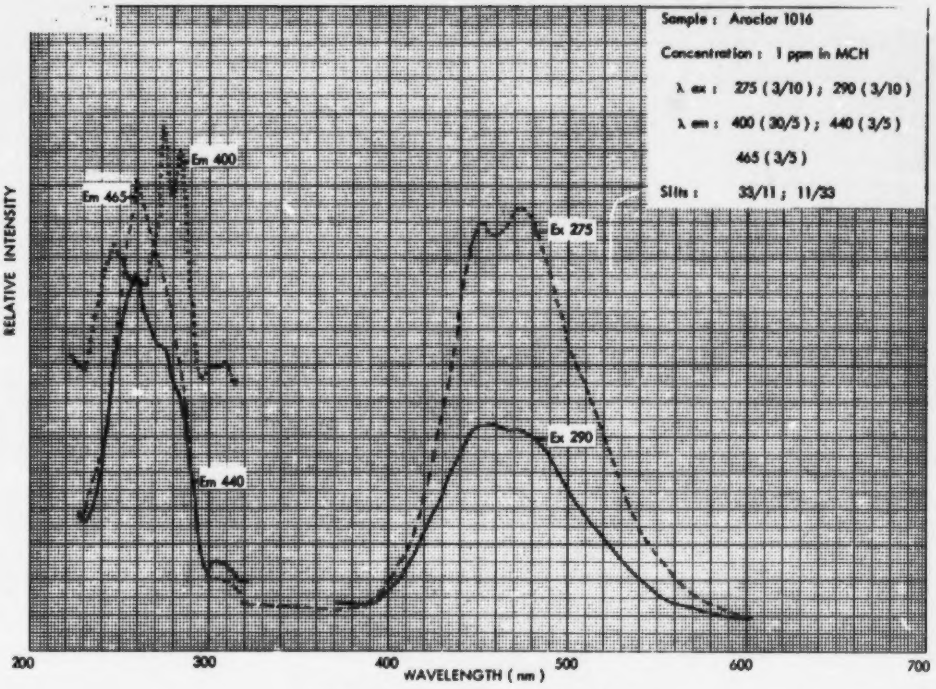


FIGURE 7. AROCLOR 1016, 1 PPM IN MCH, 77°K

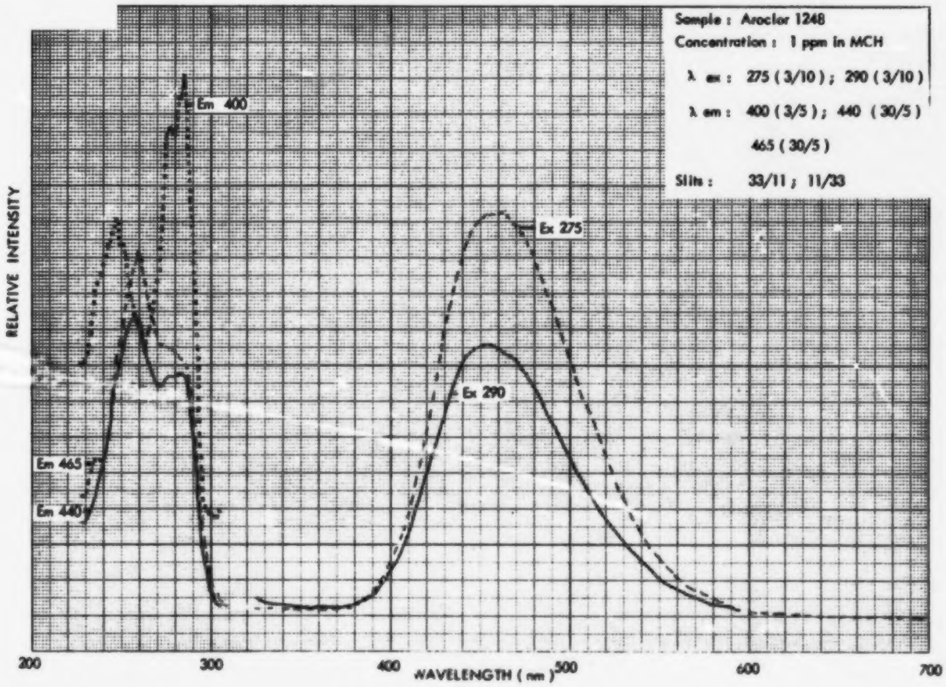


FIGURE 8. AROCLOR 1048, 1 PPM IN MCH, 77°K

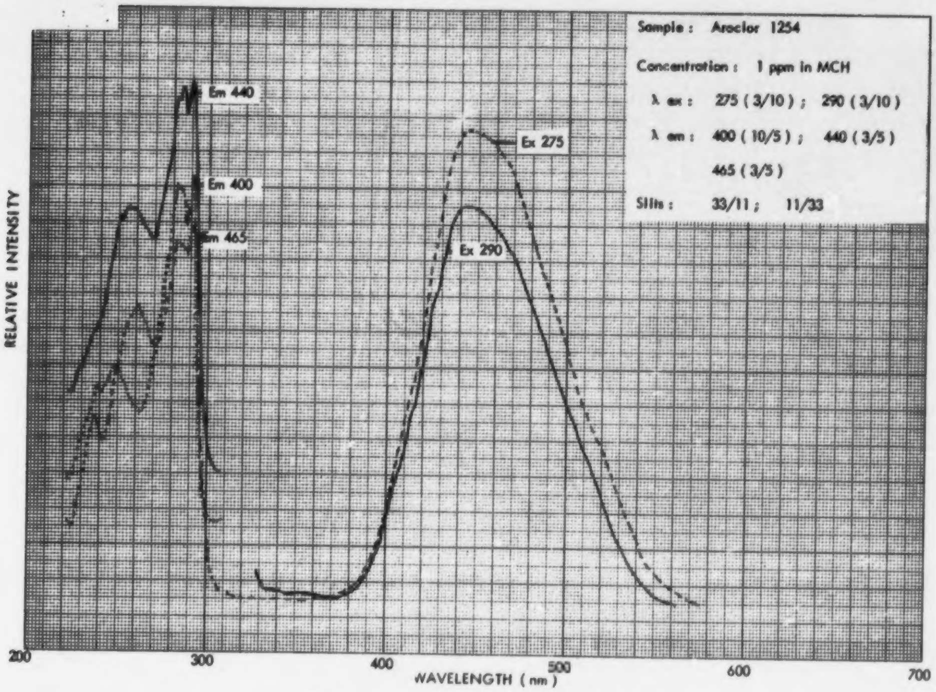


FIGURE 9. AROCLOR 1254, 1 PPM IN MCH, 77°K

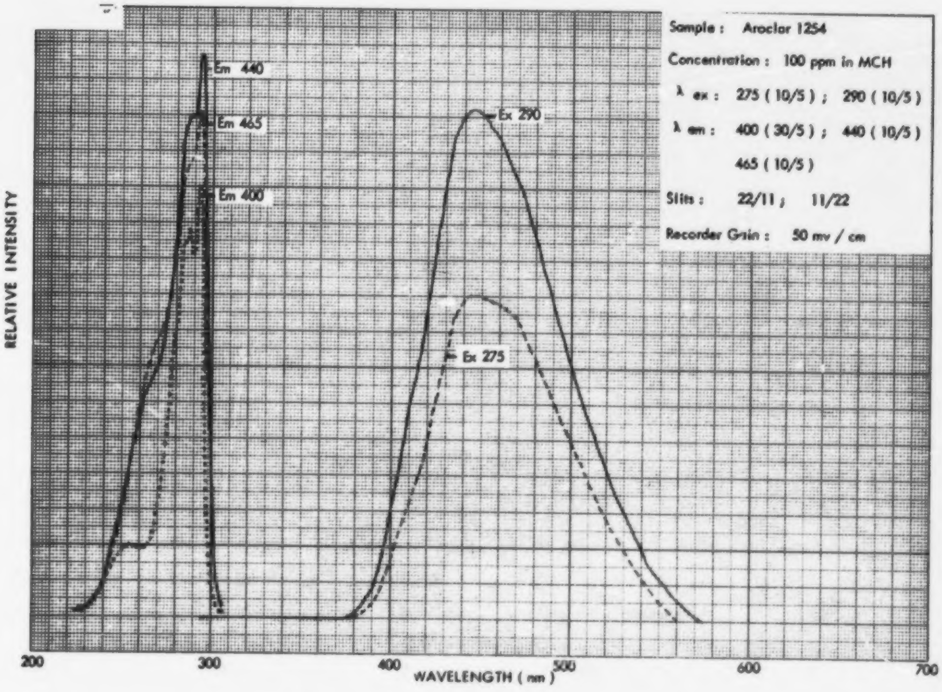


FIGURE 10. AROCLOR 1254, 100 PPM IN MCH, 77°K

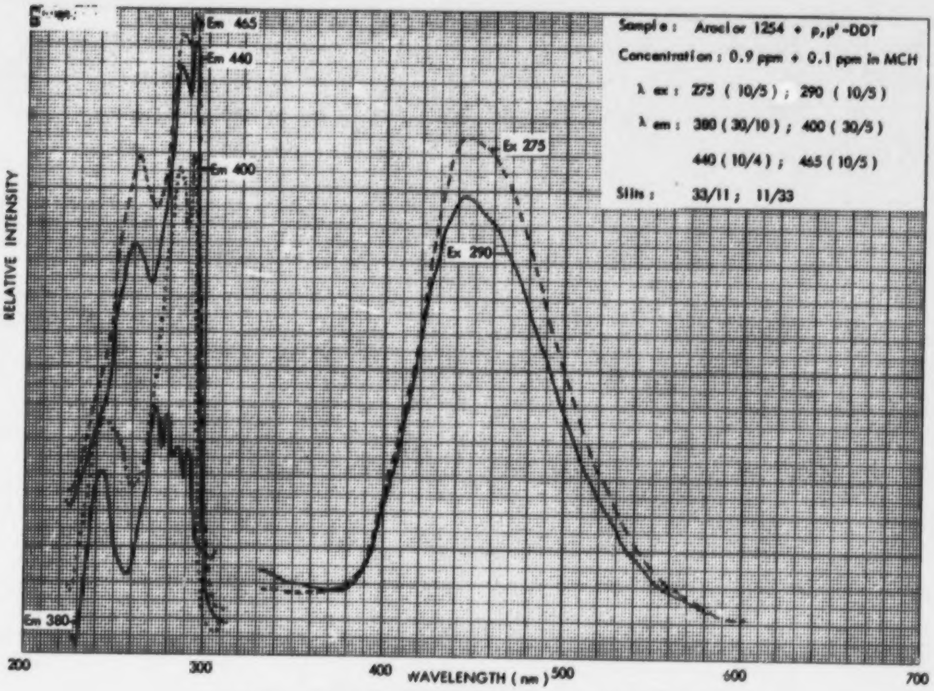


FIGURE 11. AROCLOR 1254 (0.9 PPM) + P, P'-DDT (0.1 PPM) IN MCH, 77°K

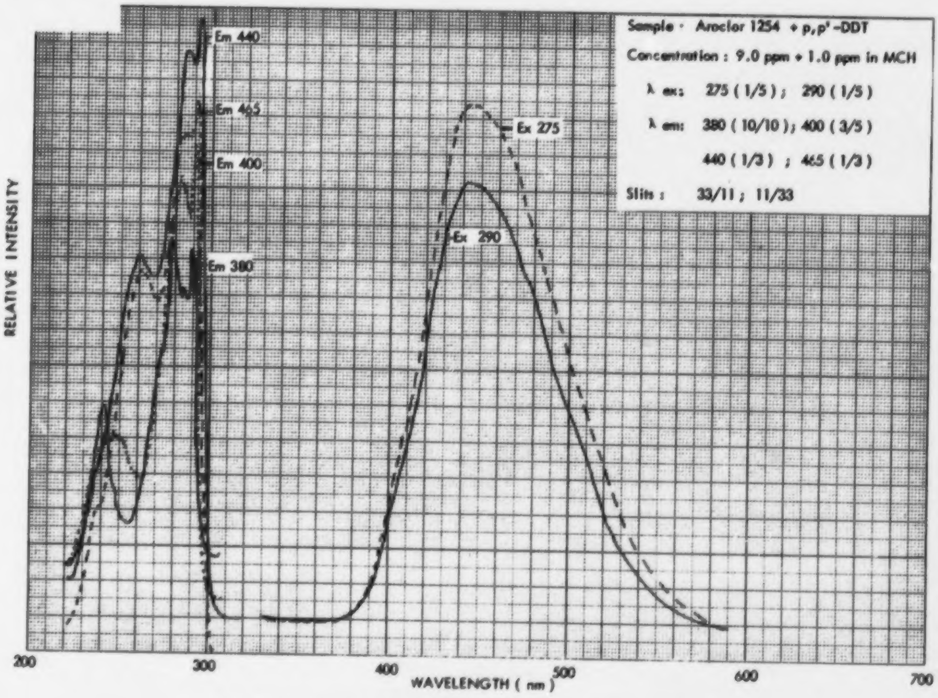


FIGURE 12. AROCLOR 1254 (9 PPM) + P, P'-DDT (1 PPM) IN MCH, 77°K

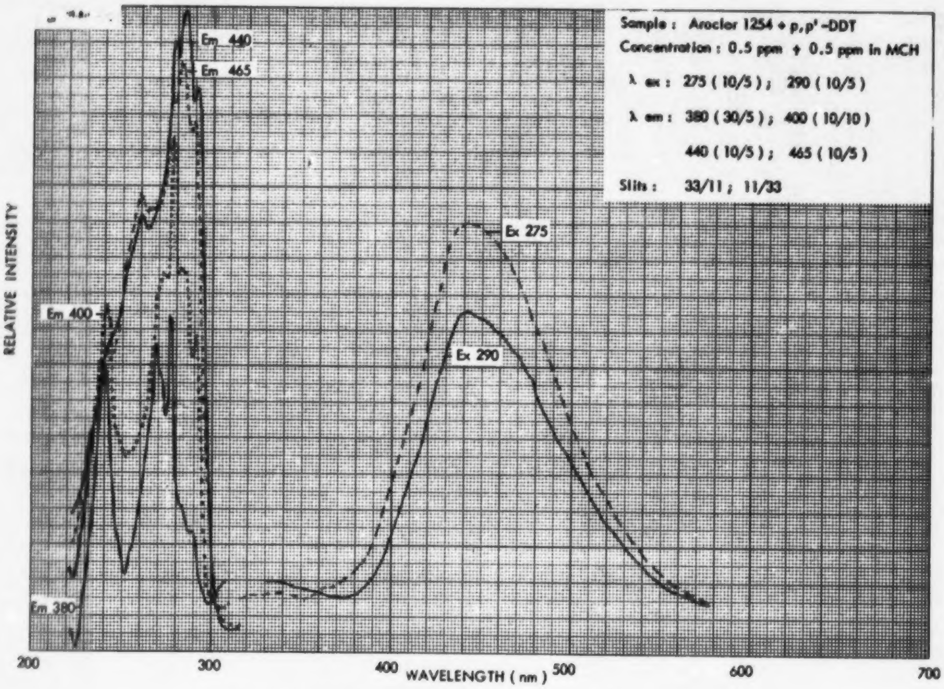


FIGURE 13. AROCLOR 1254 (0.5 PPM) + P, P'-DDT (0.5 PPM) IN MCH, 77°K

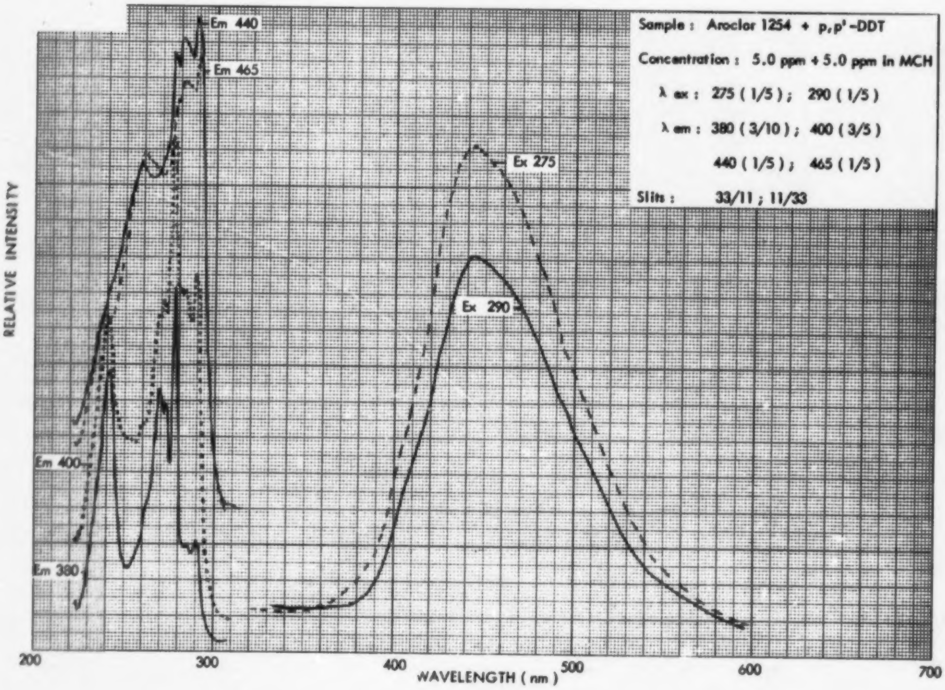


FIGURE 14. AROCLOR 1254 (5 PPM) + P, P'-DDT (5 PPM) IN MCH, 77°K

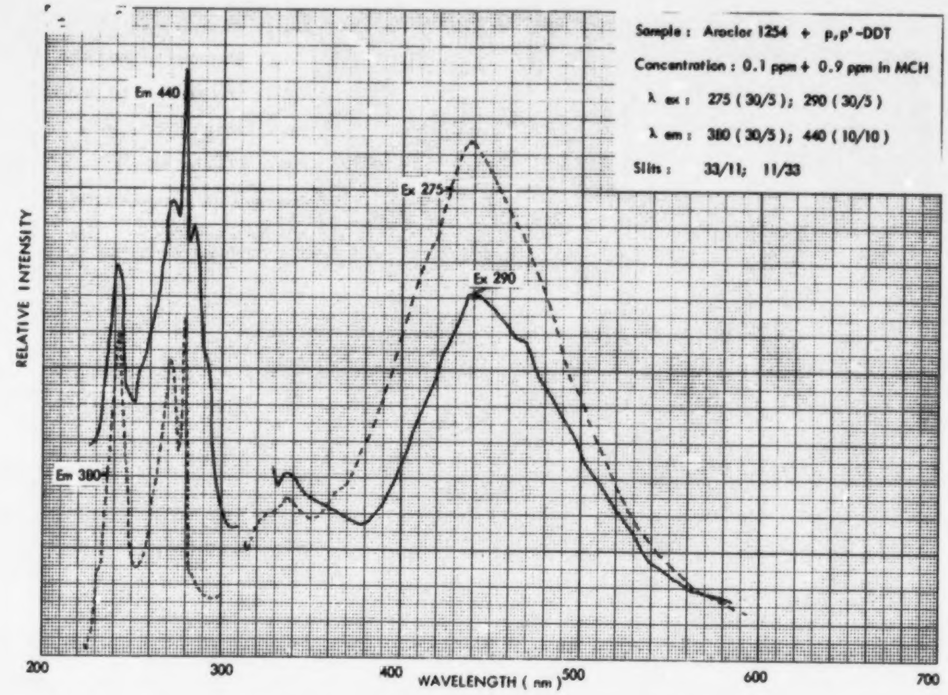


FIGURE 15. AROCLOR 1254 (0.1 PPM) + P, P'-DDT (0.9 PPM) in MCH, 77°K

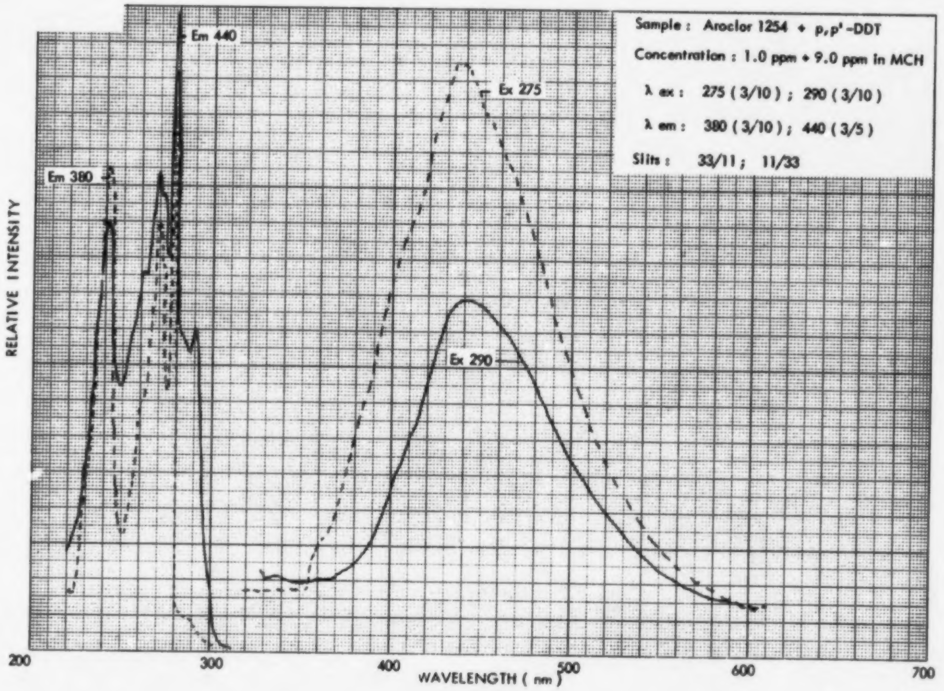


FIGURE 16. AROCLOR 1254 (1 PPM) + P, P'-DDT (9 PPM) IN MCH, 77°K

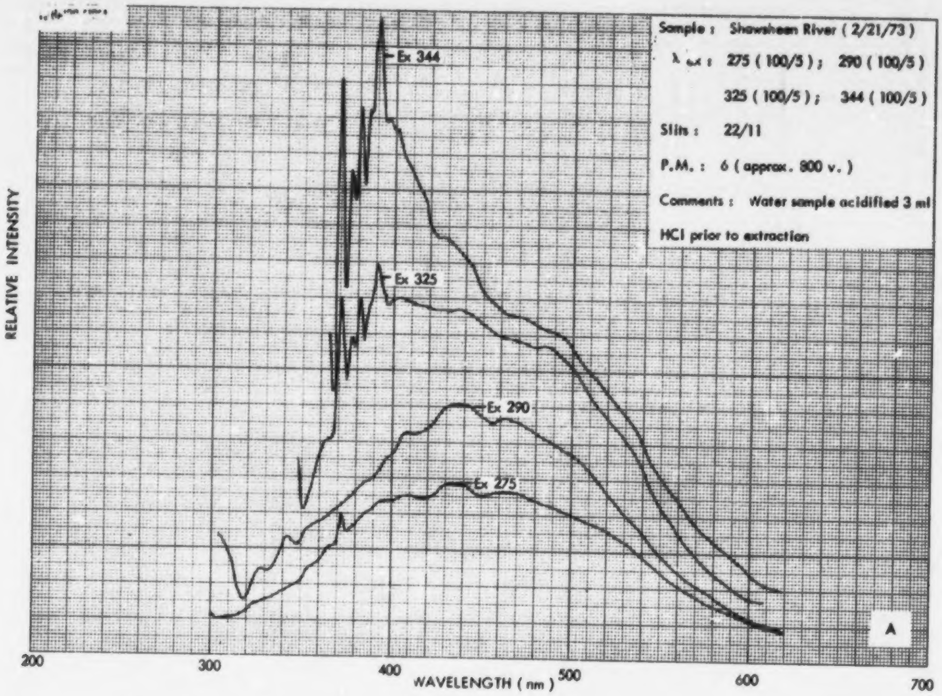
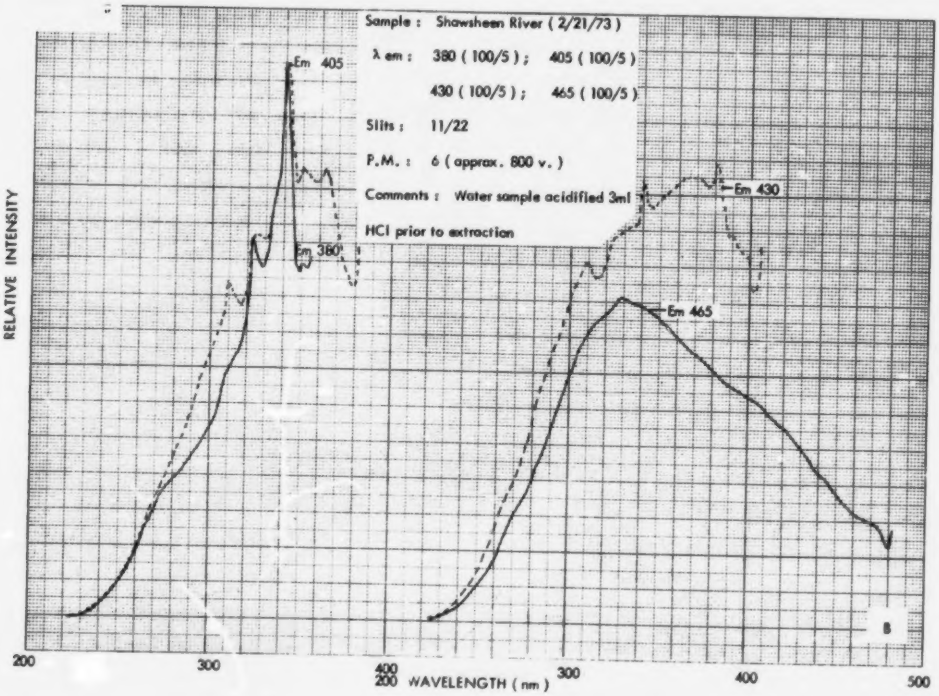


FIGURE 17A, B. SHAWSHEEN RIVER (2/21/73) EXTRACT IN MCH, 77°K (ACIDIFIED SAMPLE)



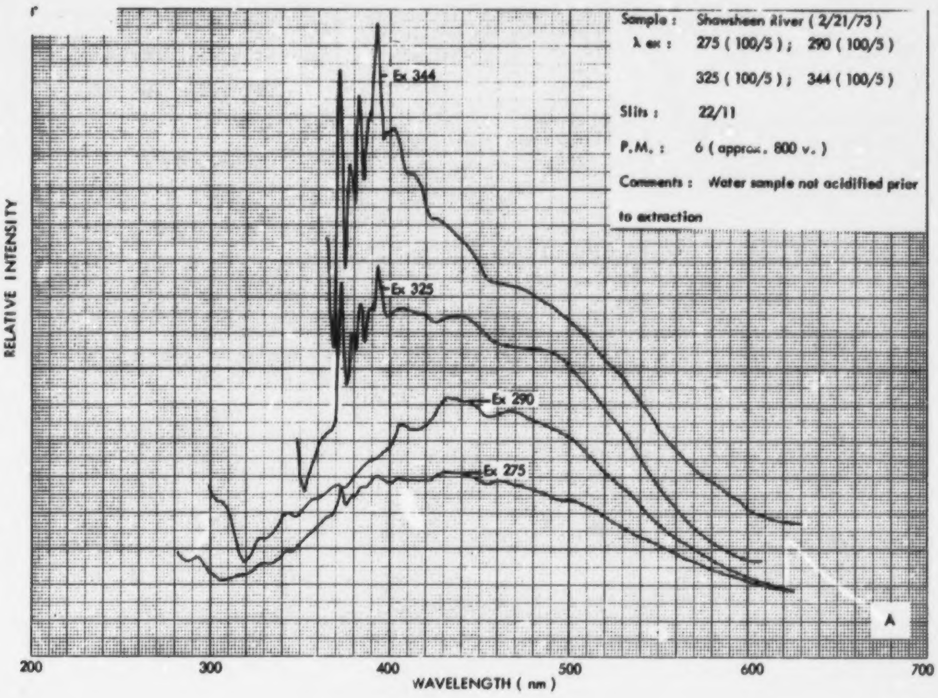
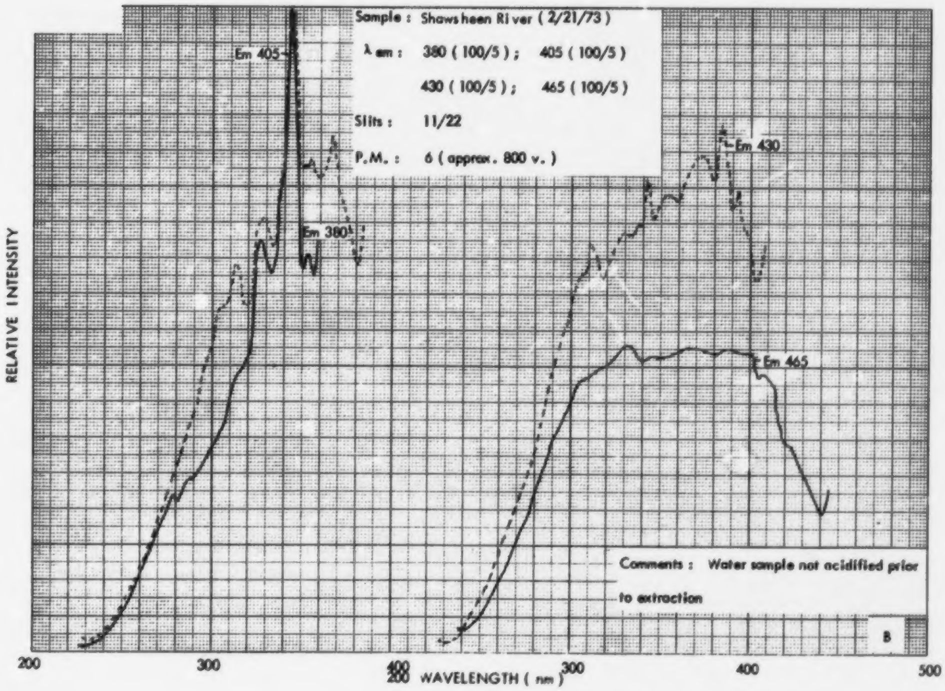


FIGURE 18A, B. SHAWSHEEN RIVER (2/21/73) EXTRACT IN MCH, 77°K (SAMPLE NOT ACIDIFIED)



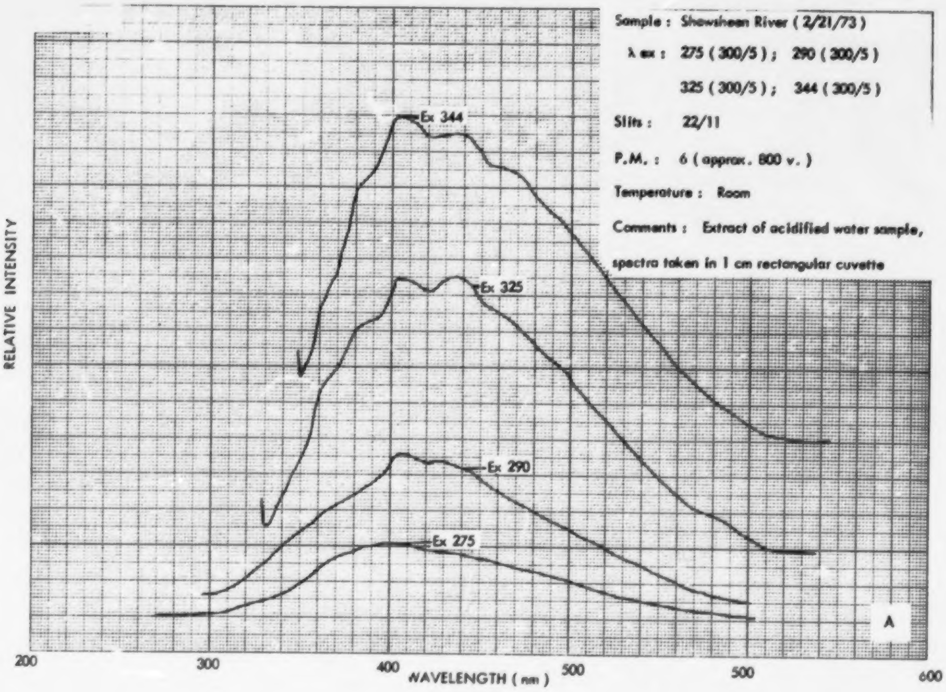
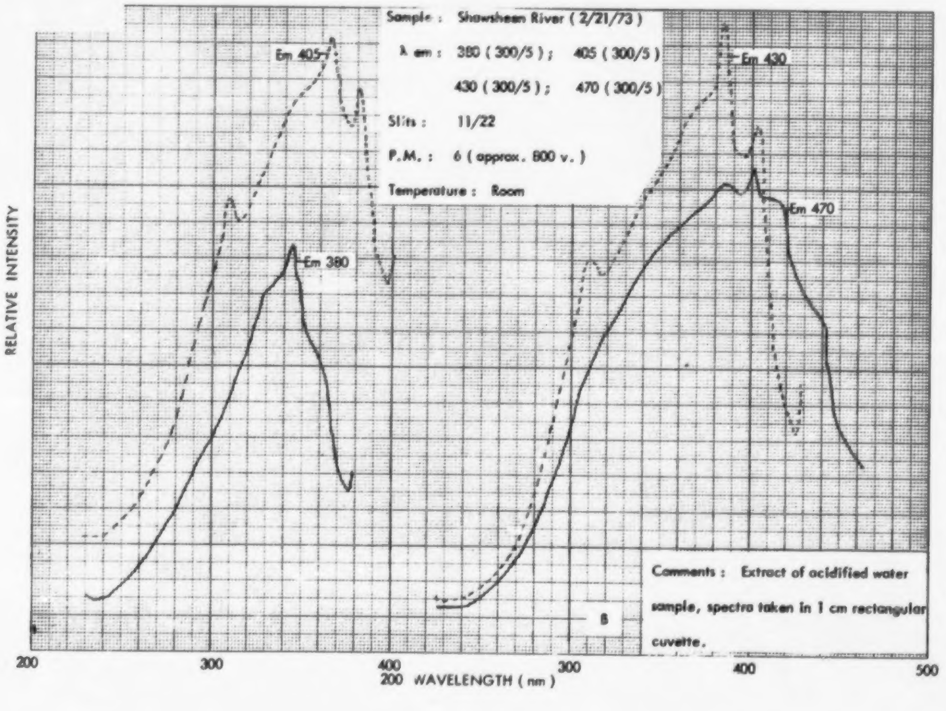


FIGURE 19A, B. SHAWSHEEN RIVER (2/21/73) EXTRACT IN MCH, ROOM TEMPERATURE (ACIDIFIED SAMPLE)



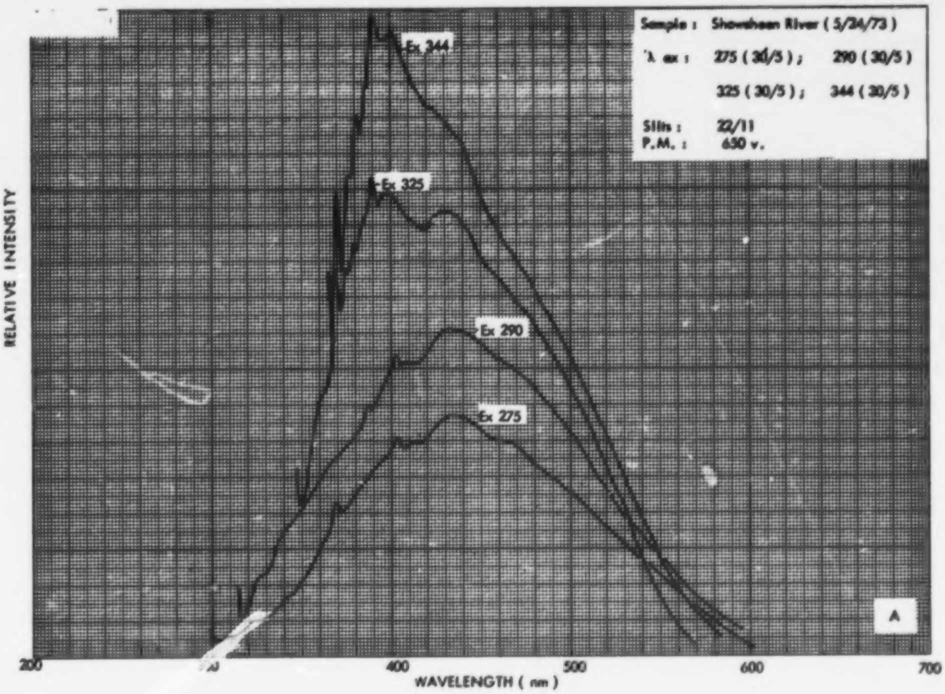
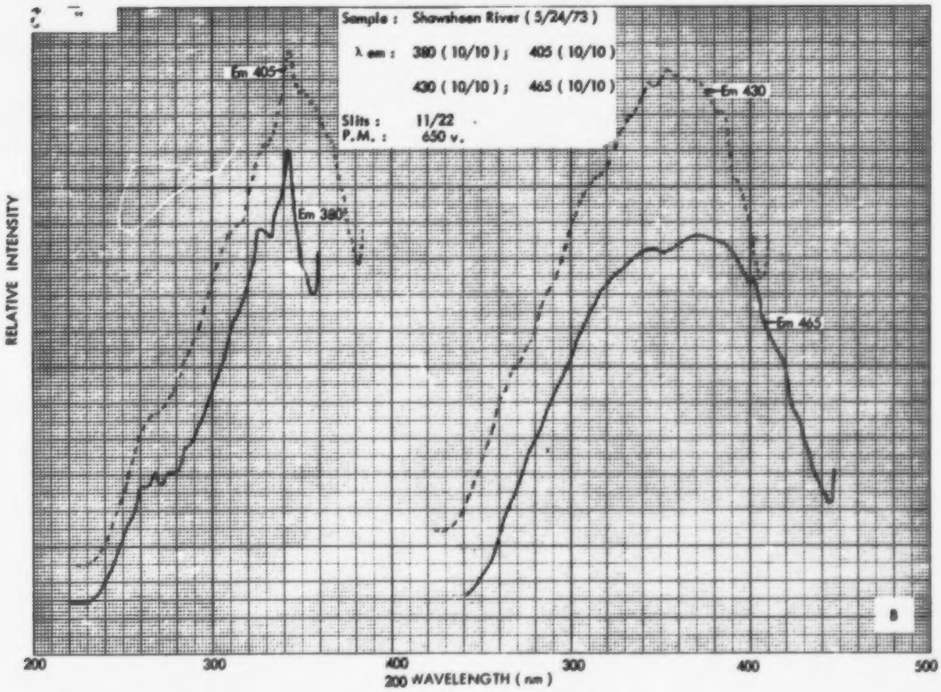


FIGURE 20A, B. SHAWSHEEN RIVER (5/24/73) EXTRACT IN MCH, 77°K



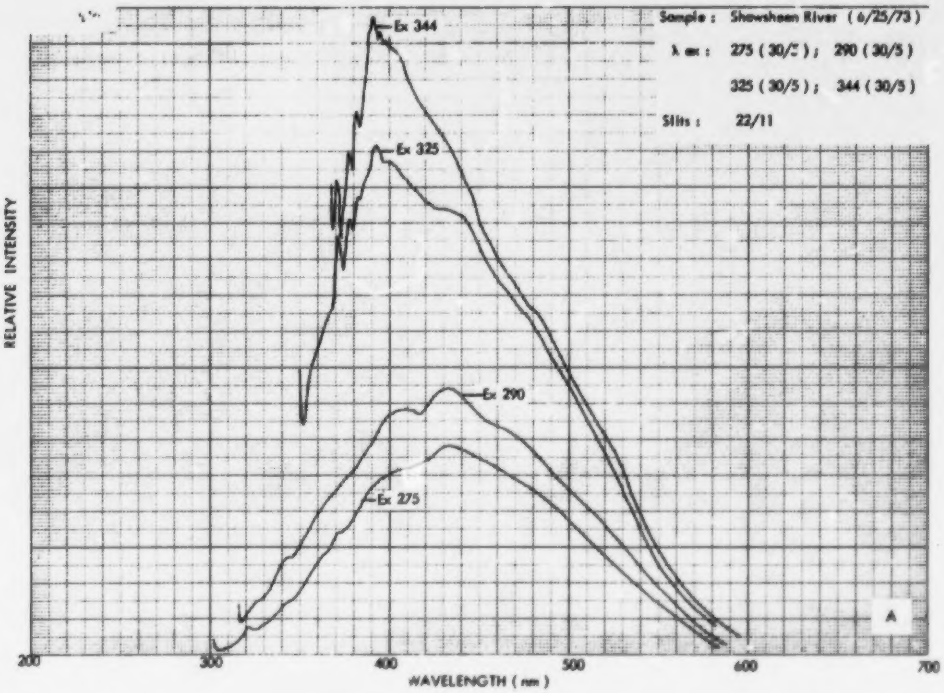
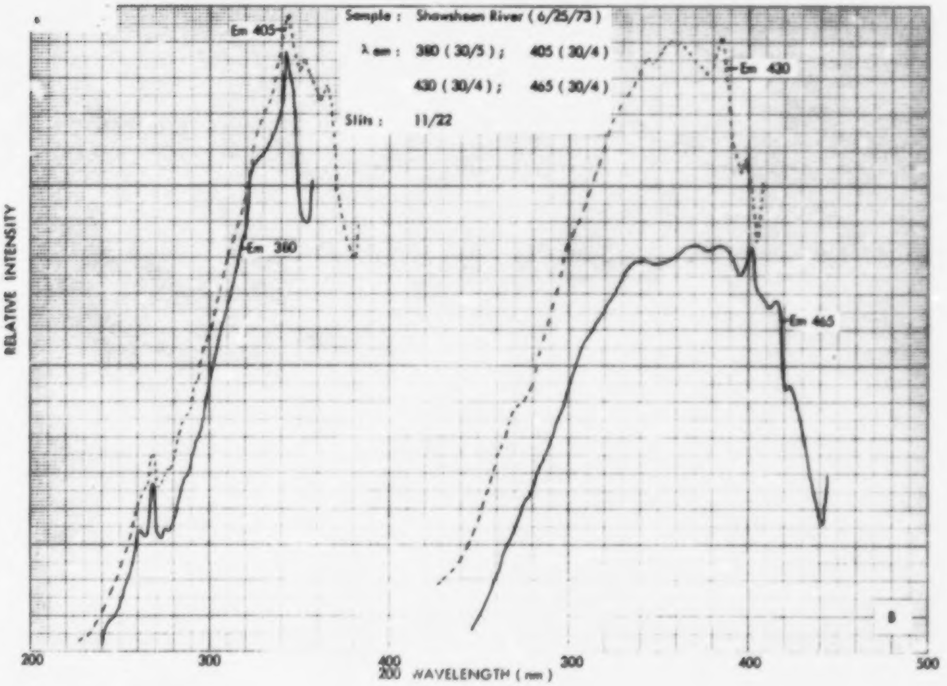


FIGURE 21A, B. SHAWSHEEN RIVER (6/25/73) EXTRACT IN MCH, 77°K



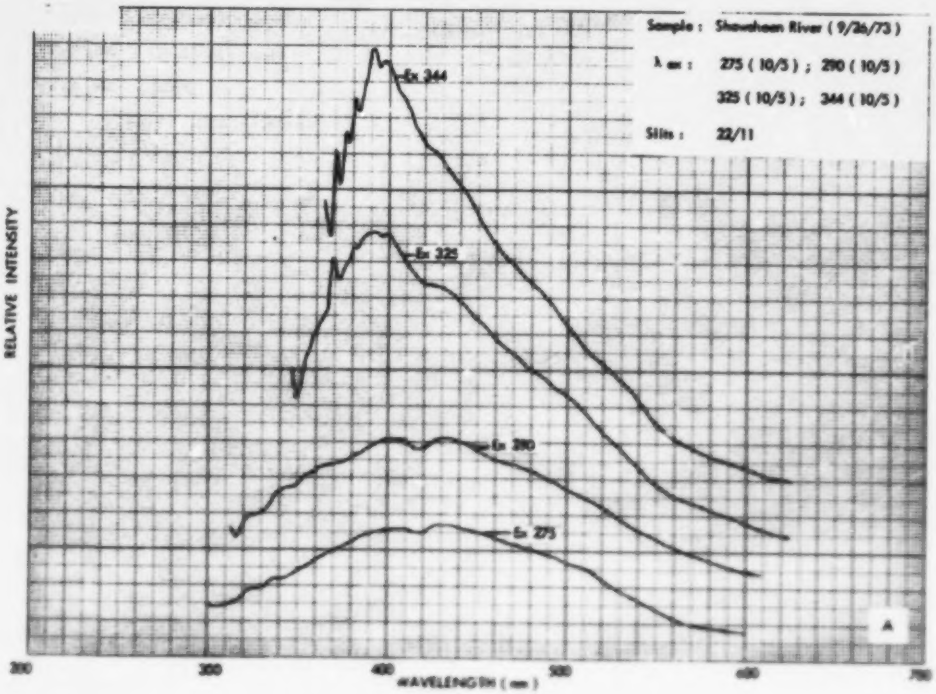
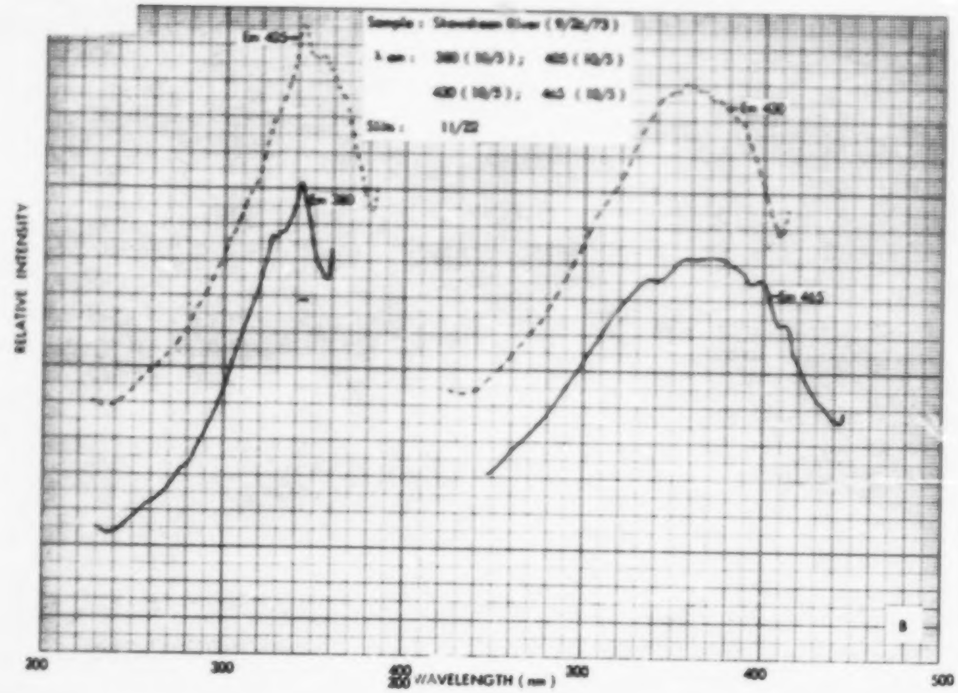


FIGURE 22A, B. SHAWSHEEN RIVER (9/26/73) EXTRACT IN MCH, 77°K



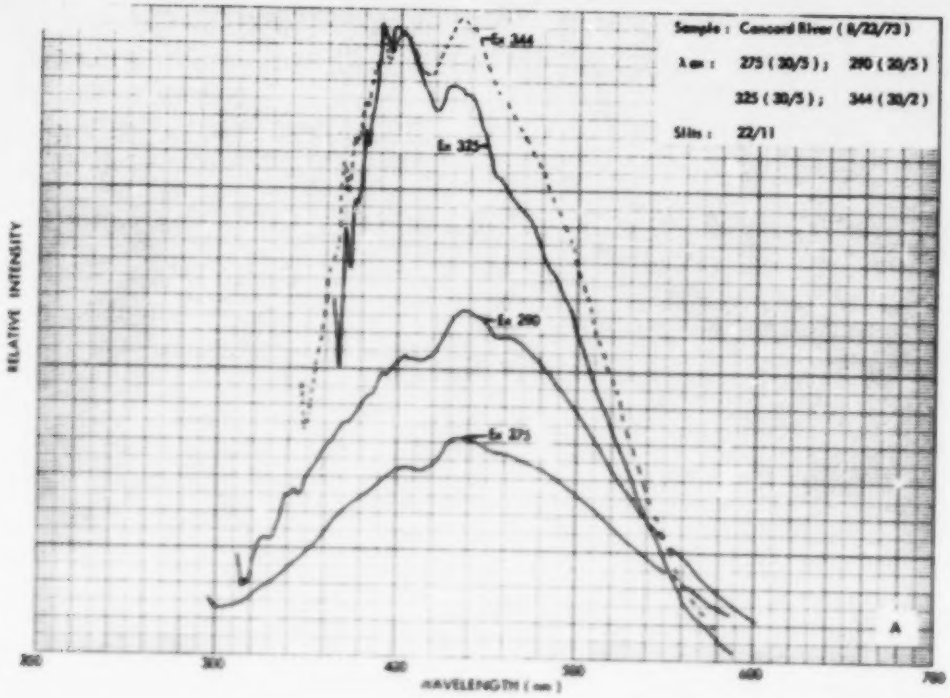
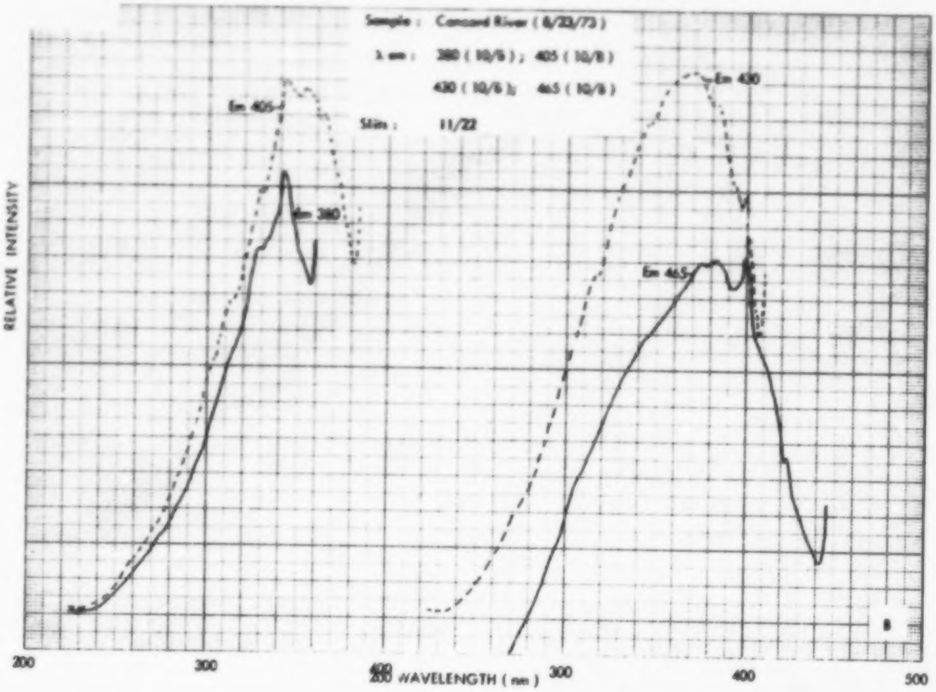


FIGURE 23A, B. CONCORD RIVER (8/23/73) EXTRACT IN MCH, 77°K



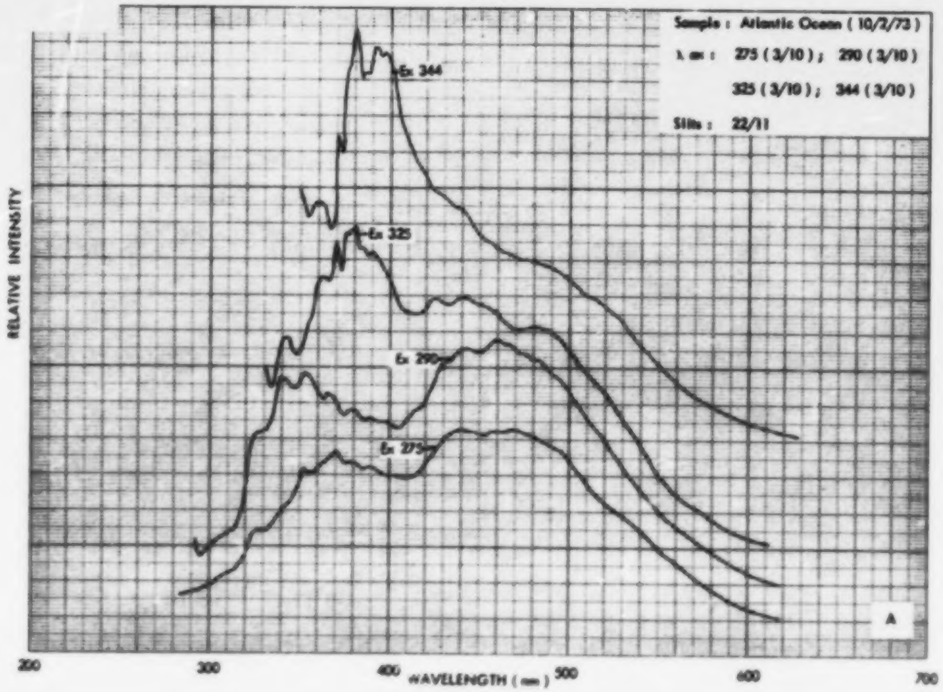
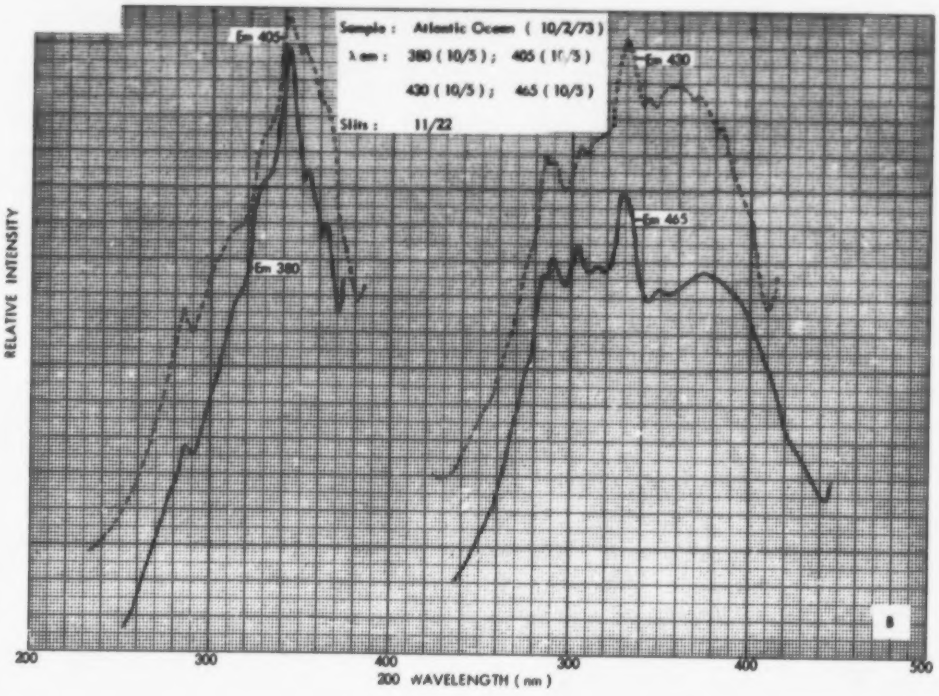


FIGURE 24A, B. ATLANTIC OCEAN (10/2/73) EXTRACT IN MCH, 77°K



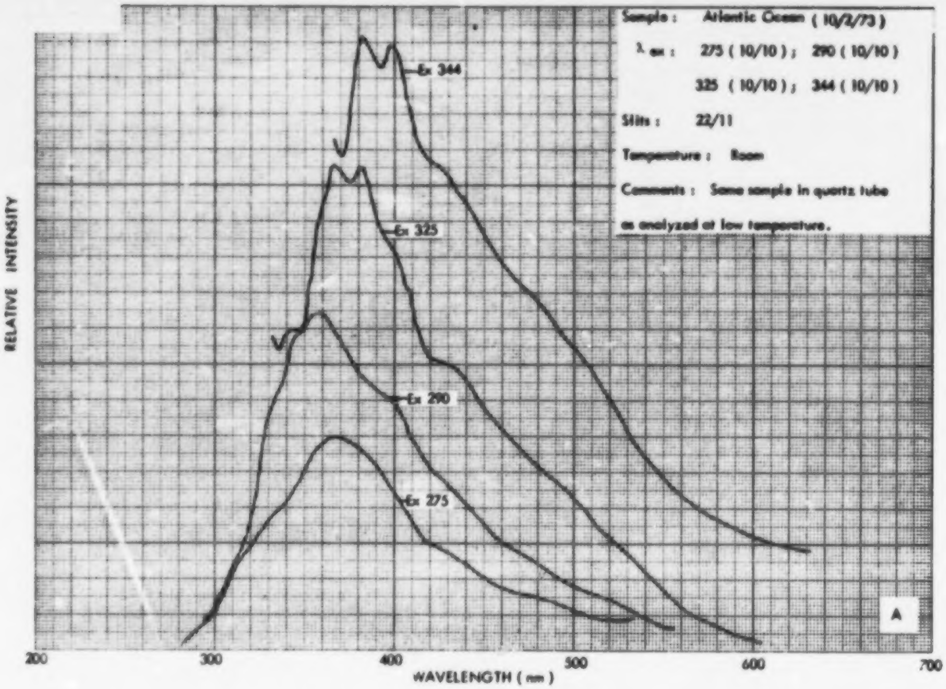
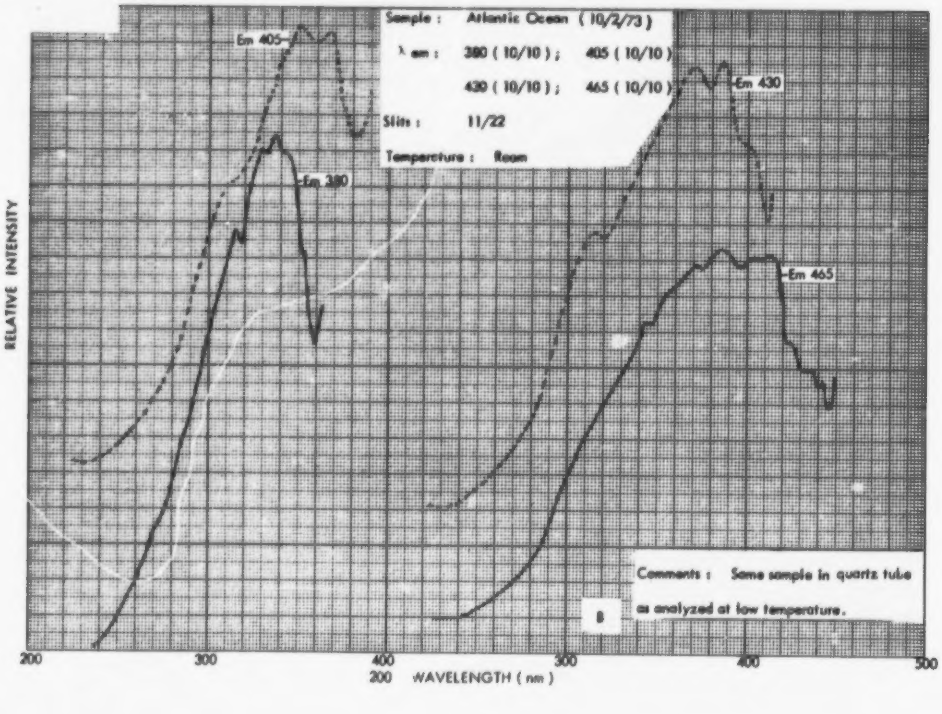


FIGURE 25A, B. ATLANTIC OCEAN (10/2/73) EXTRACT IN MCH, ROOM TEMPERATURE



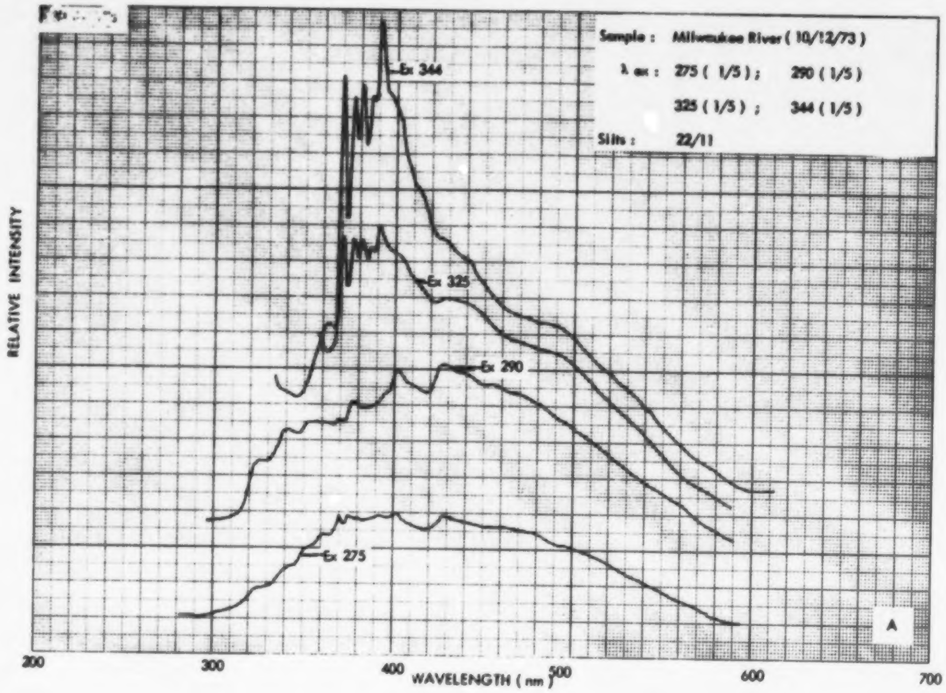
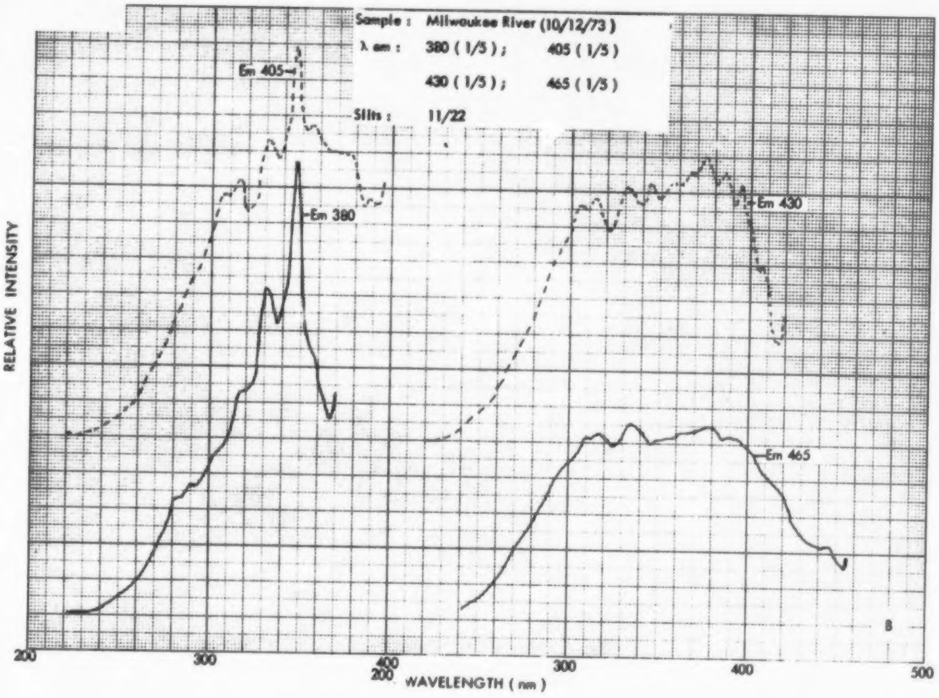


FIGURE 26A, B. MILWAUKEE RIVER (10/12/73) EXTRACT IN MCH, 77°K



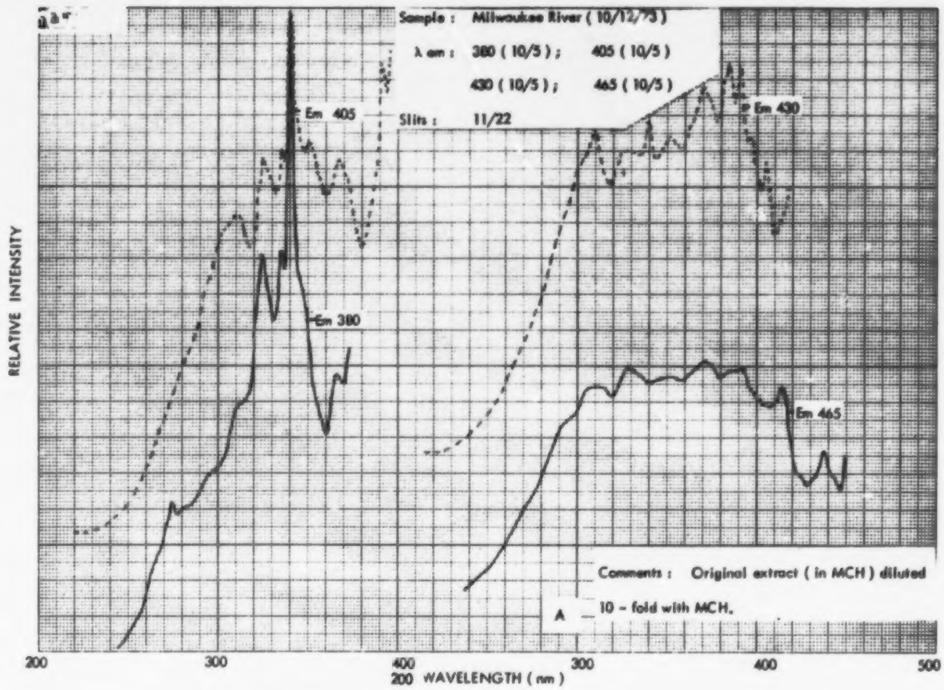
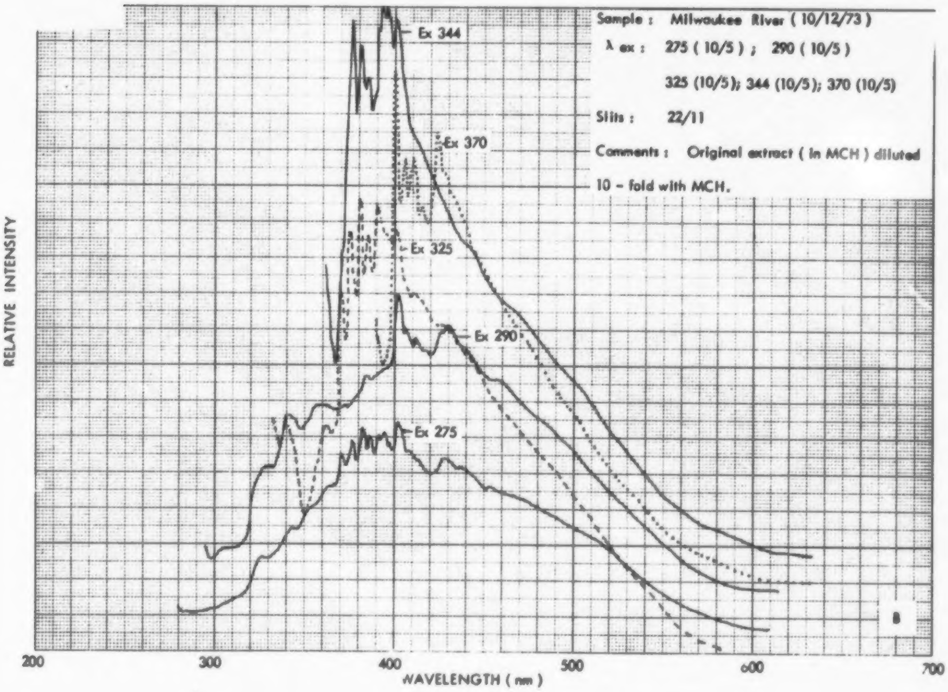


FIGURE 27A, B. MILWAUKEE RIVER (10/12/73) EXTRACT IN MCH, 77°K ORIGINAL EXTRACT DILUTED 10-FOLD WITH MCH



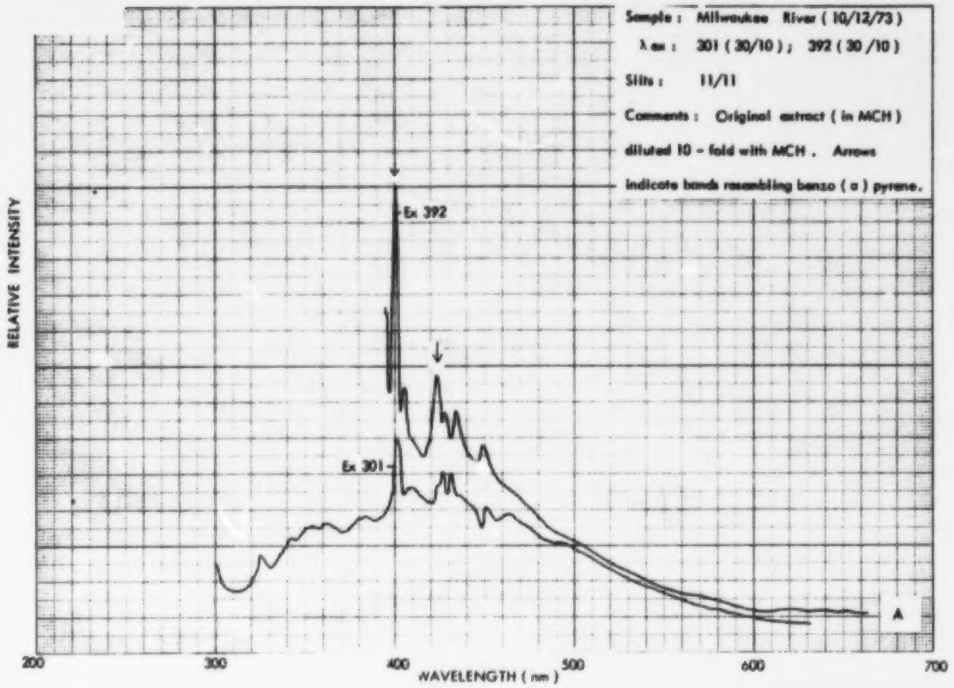
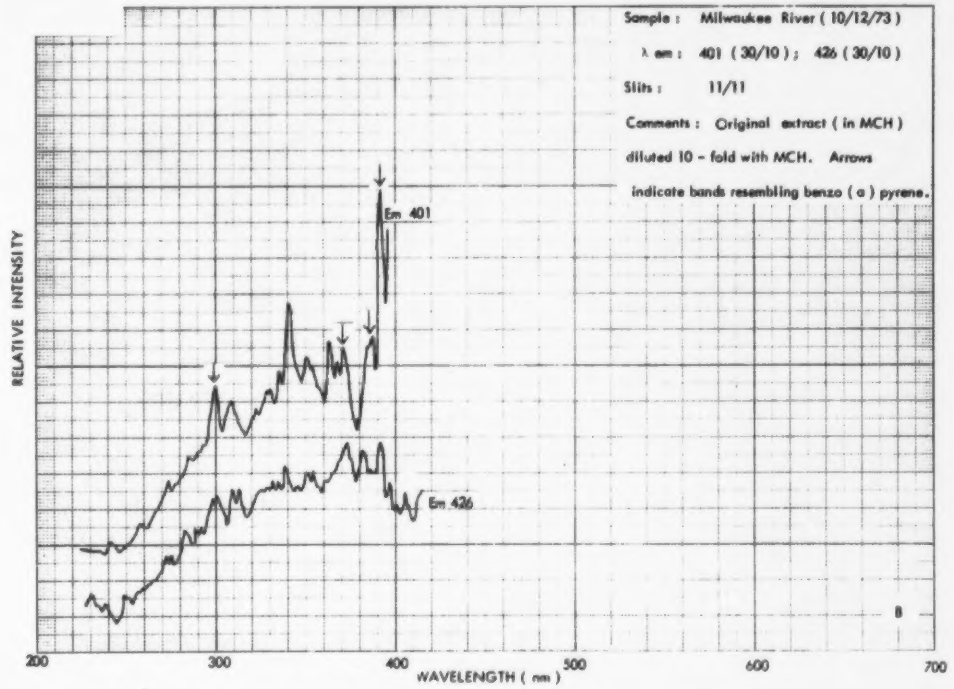


FIGURE 28A, B. MILWAUKEE RIVER (10/12/73) EXTRACT IN MCH, 77°K - DETAIL OF STRUCTURE RESEMBLING BENZO(A) PYRENE



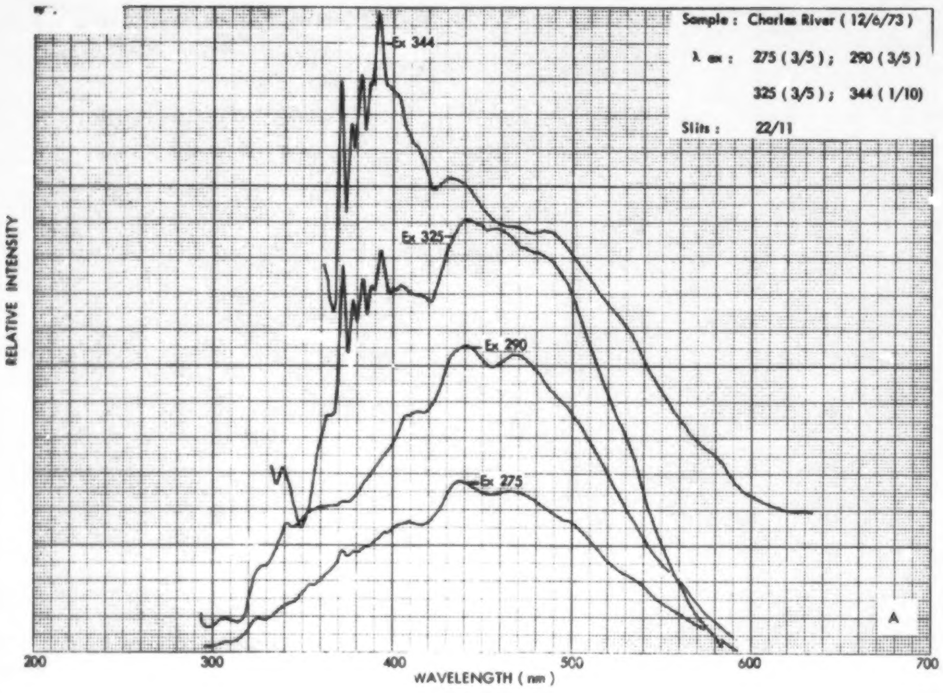
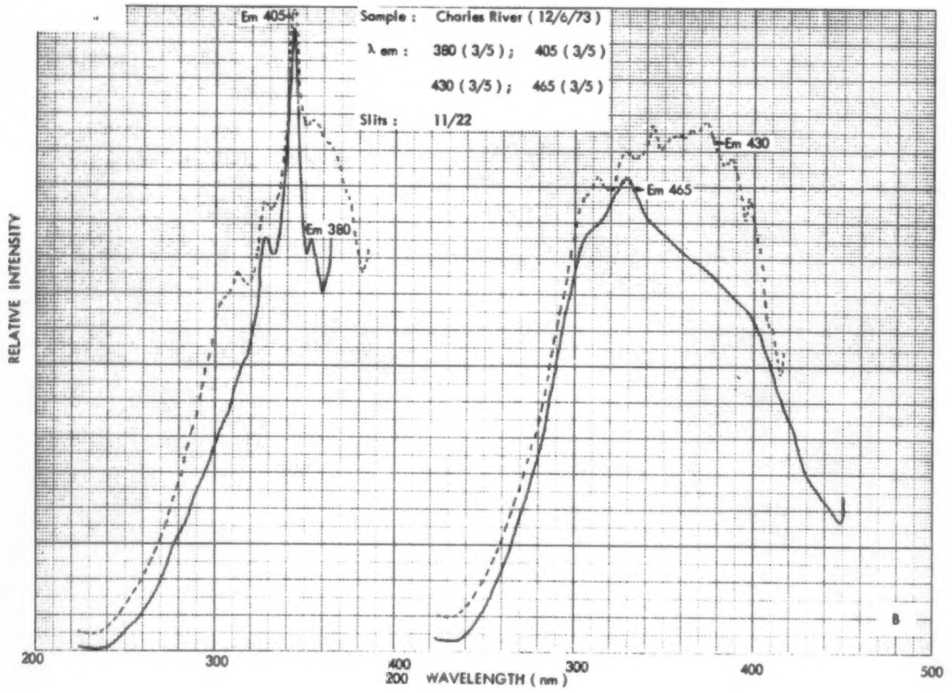


FIGURE 29A, B. CHARLES RIVER (12/6/73) EXTRACT IN MCH, 77°K



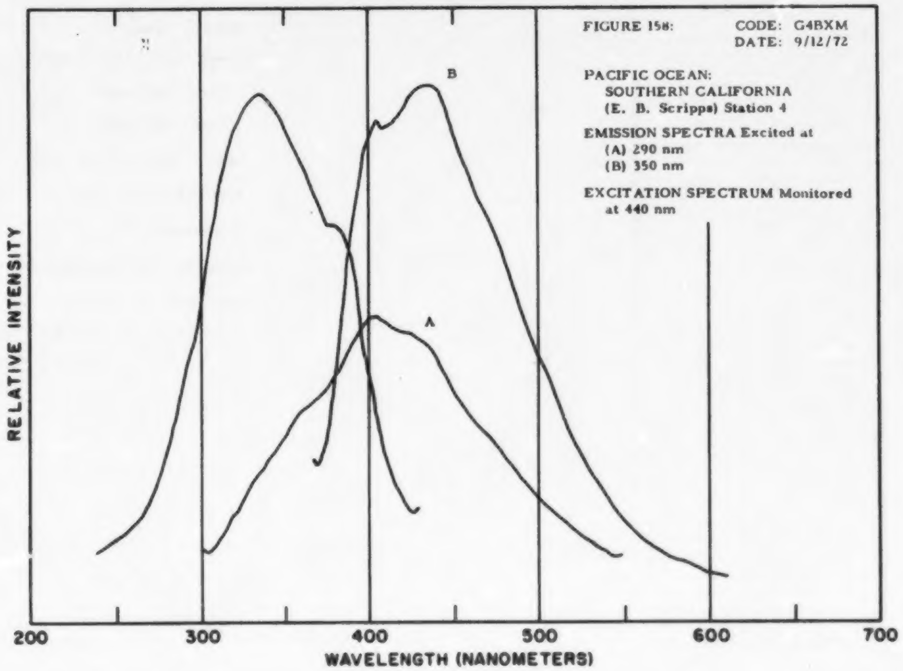


FIGURE 30. PACIFIC OCEAN (9/12/72) GELBSTOFF LUMINESCENCE AT ROOM TEMPERATURE (FROM HORNIG AND EASTWOOD, 1972)

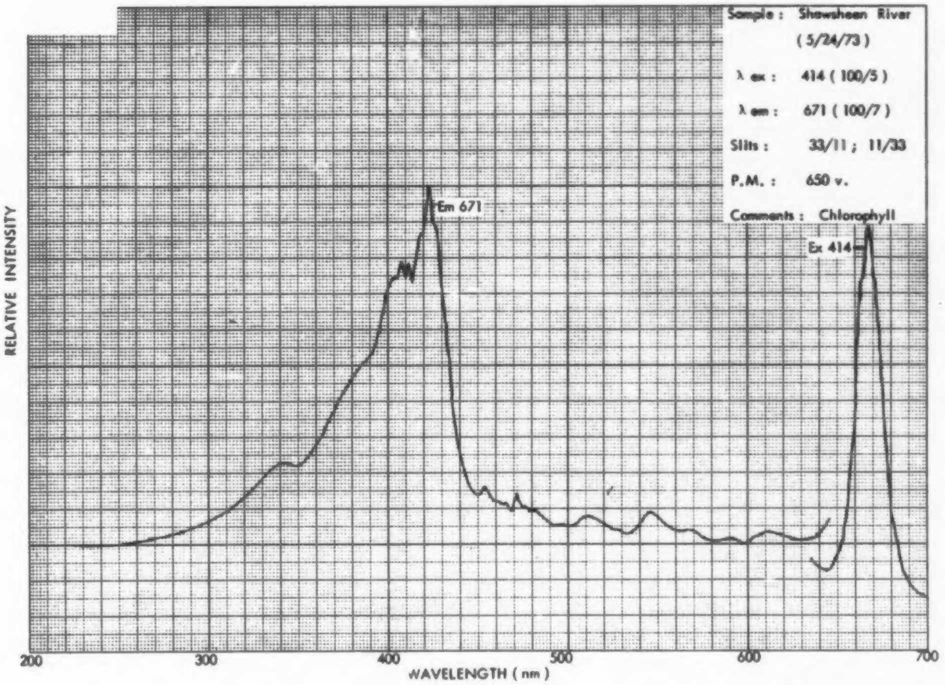


FIGURE 31. SHAWSHEEN RIVER (5/24/73) EXTRACT IN MCH, 77°K - CHLOROPHYLL LUMINESCENCE

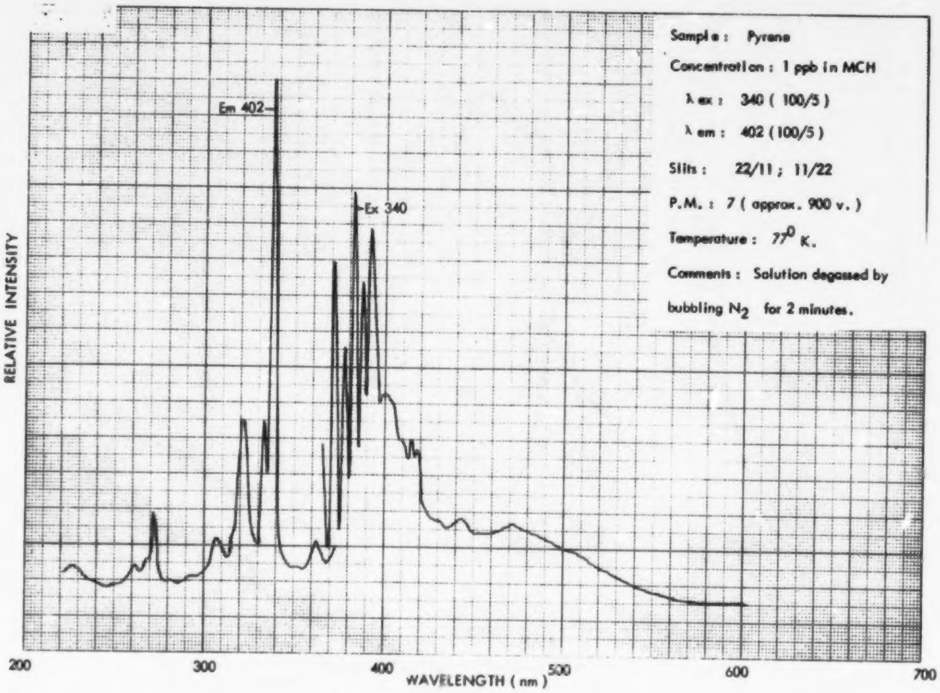


FIGURE 32. PYRENE, 1 PPB IN MCH, 77°K

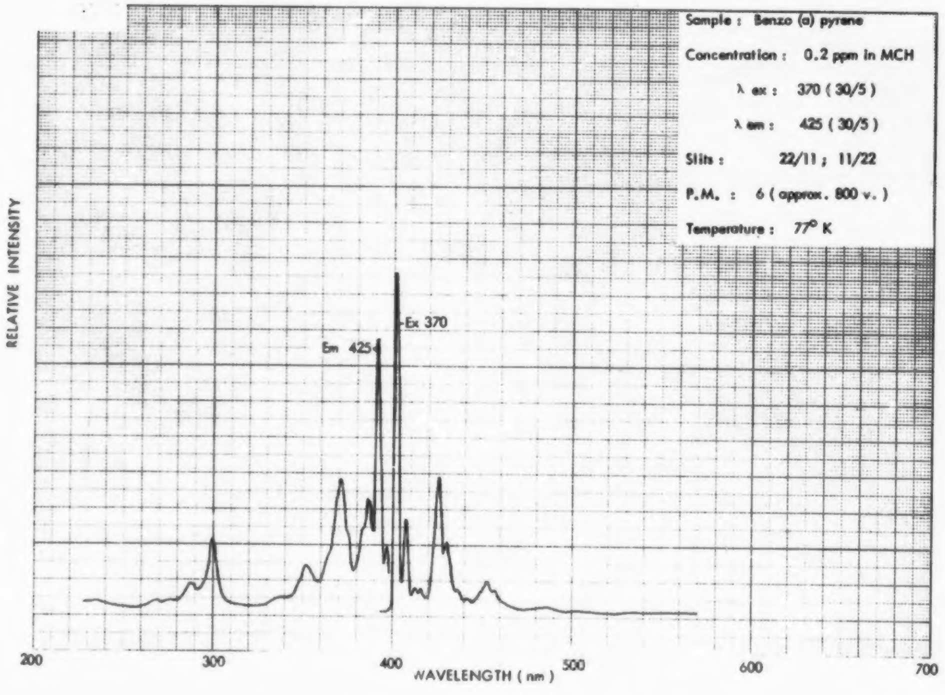


FIGURE 33. BENZO(A)PYRENE, 0.2 PPM IN MCH, 77°K

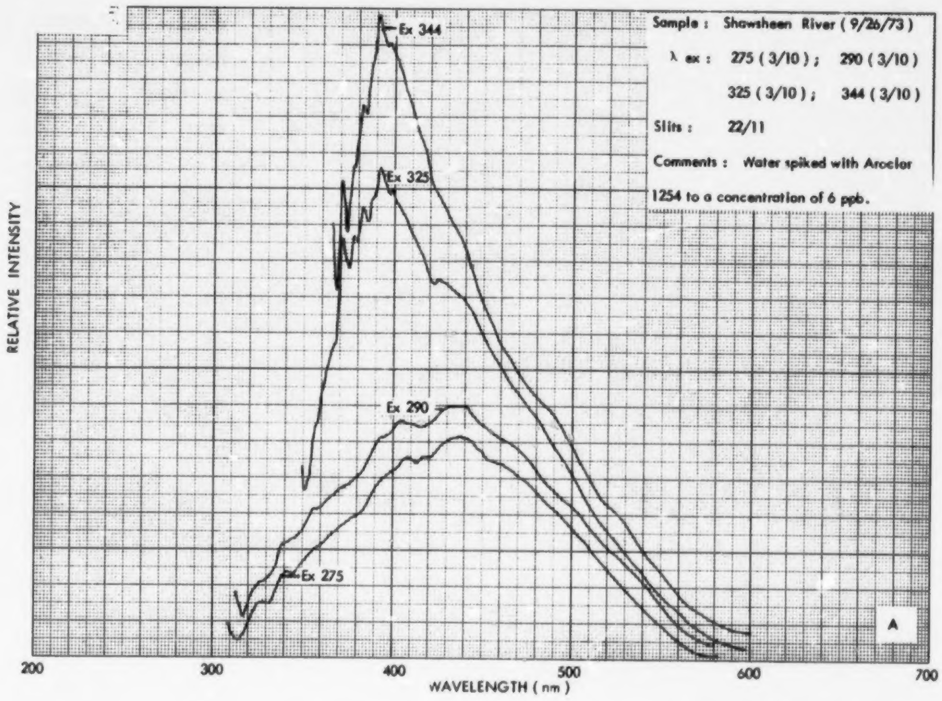
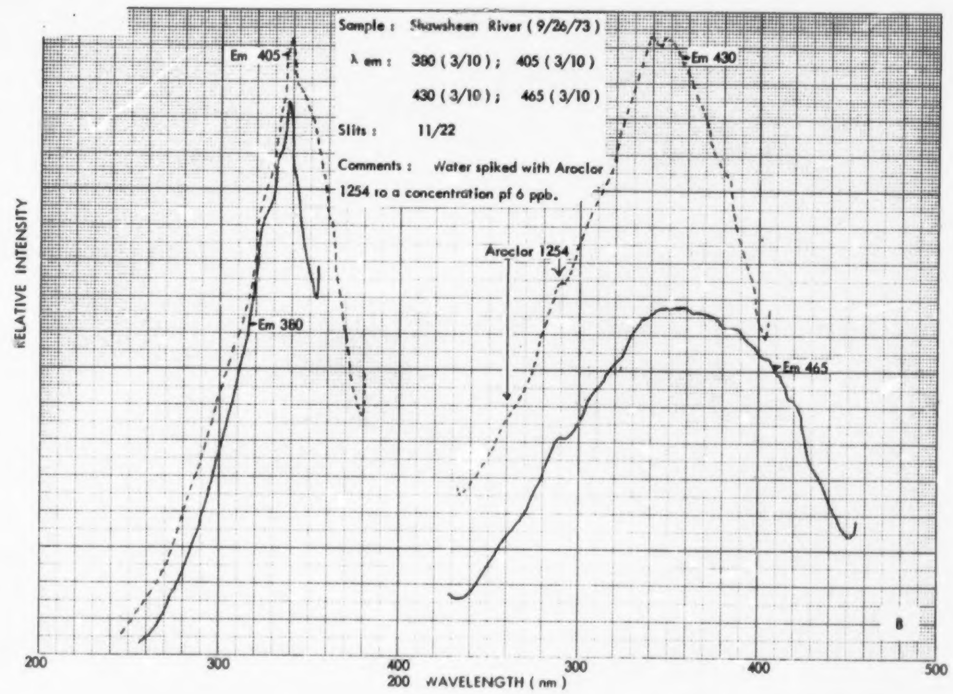


FIGURE 34A, B. SHAWSHEEN RIVER (9/26/73) EXTRACT OF WATER DOPED WITH 6 PPB AROCLOR 1254



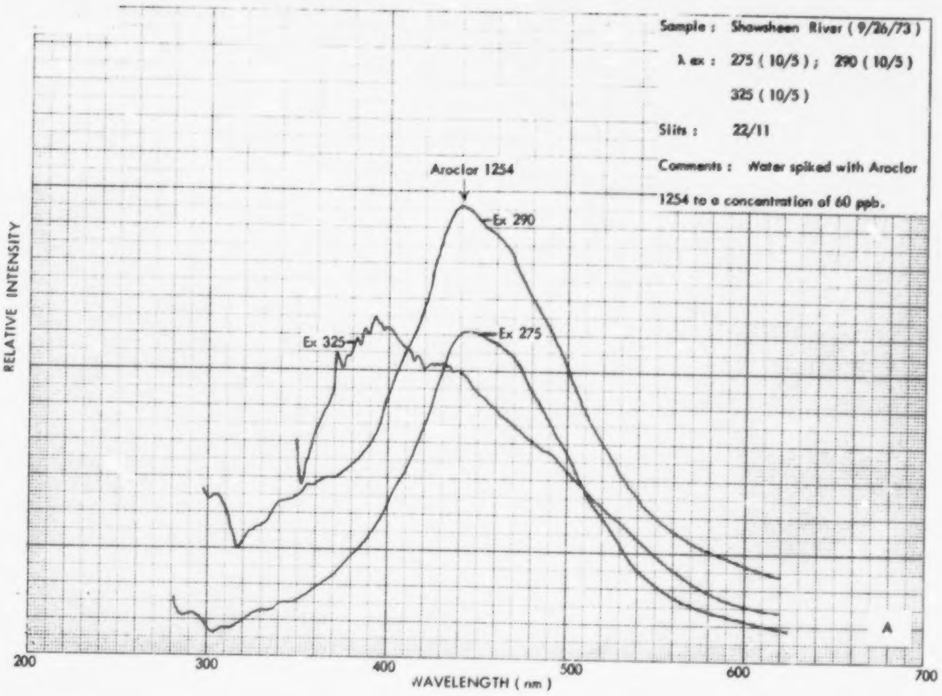
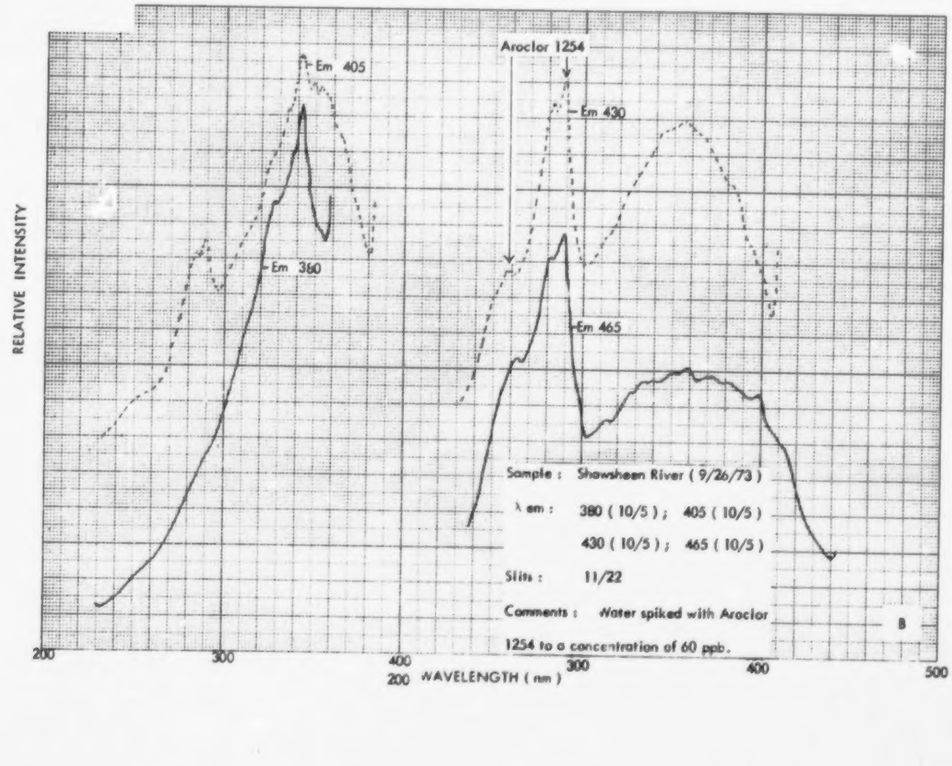


FIGURE 35A, B. SHAWSHEEN RIVER (9/26/73) EXTRACT OF WATER DOPED WITH 60 PPB AROCLOR 1254



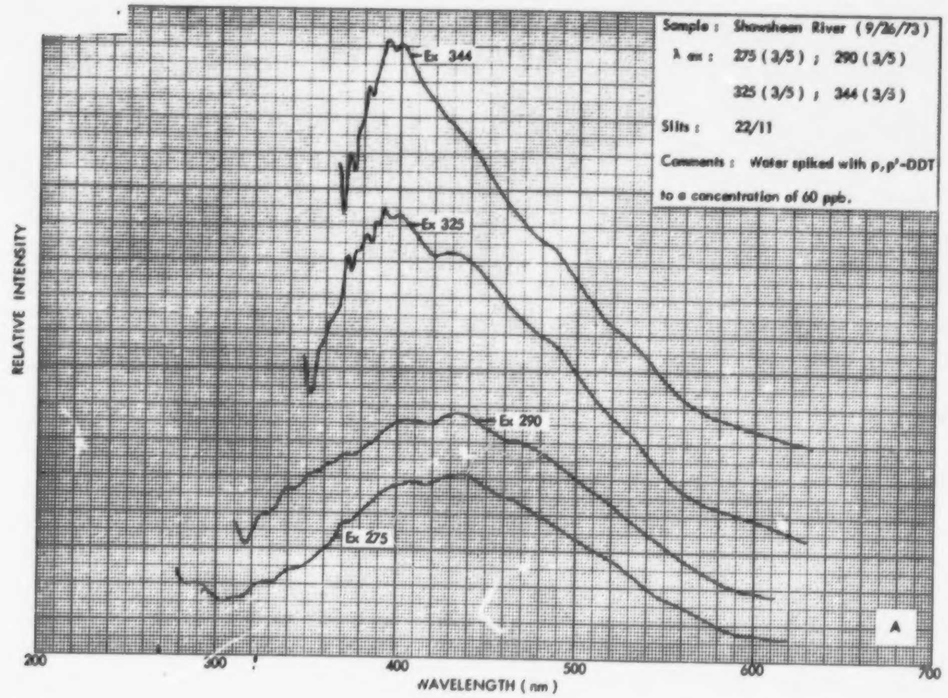
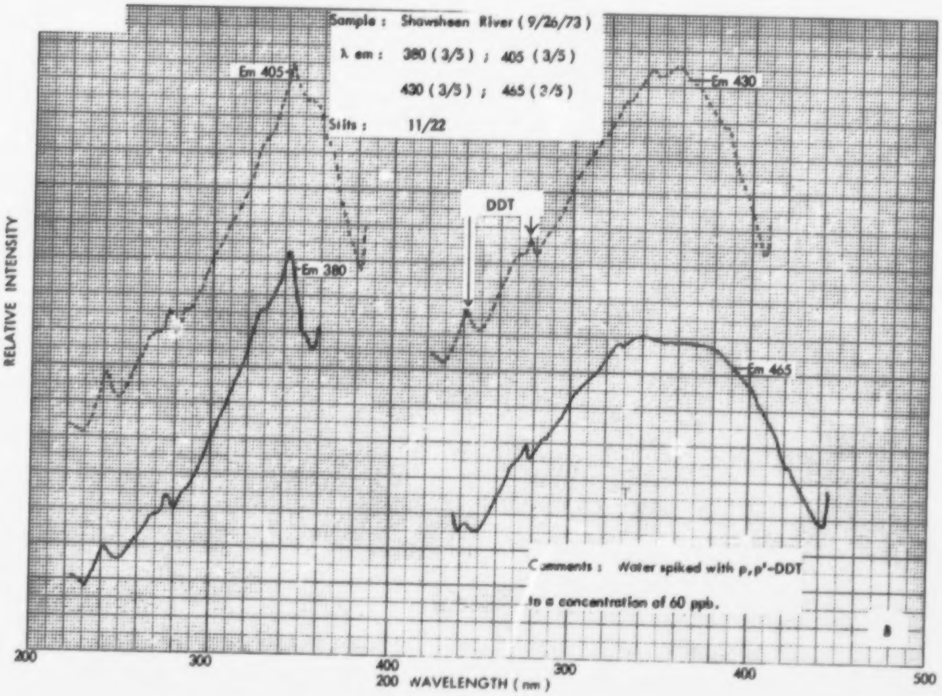


FIGURE 36A, B. SHAWSHEEN RIVER (9/26/73) EXTRACT OF WATER DOPED WITH 60 PPB P, P'-DDT



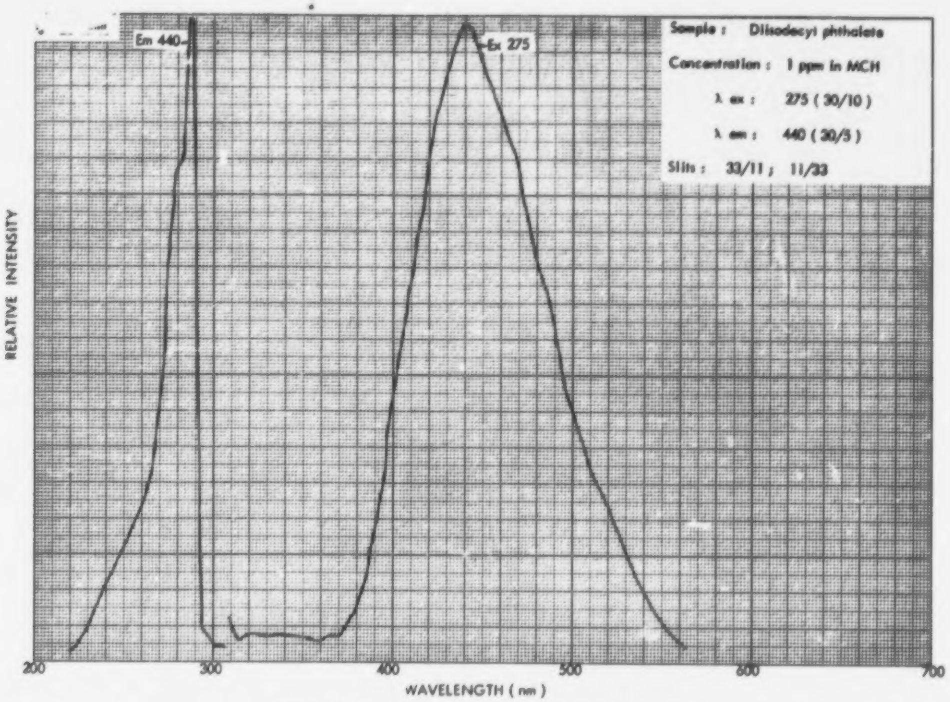


FIGURE 37. DIISODECYL PHTHALATE, 1 PPM IN MCH, 77°K

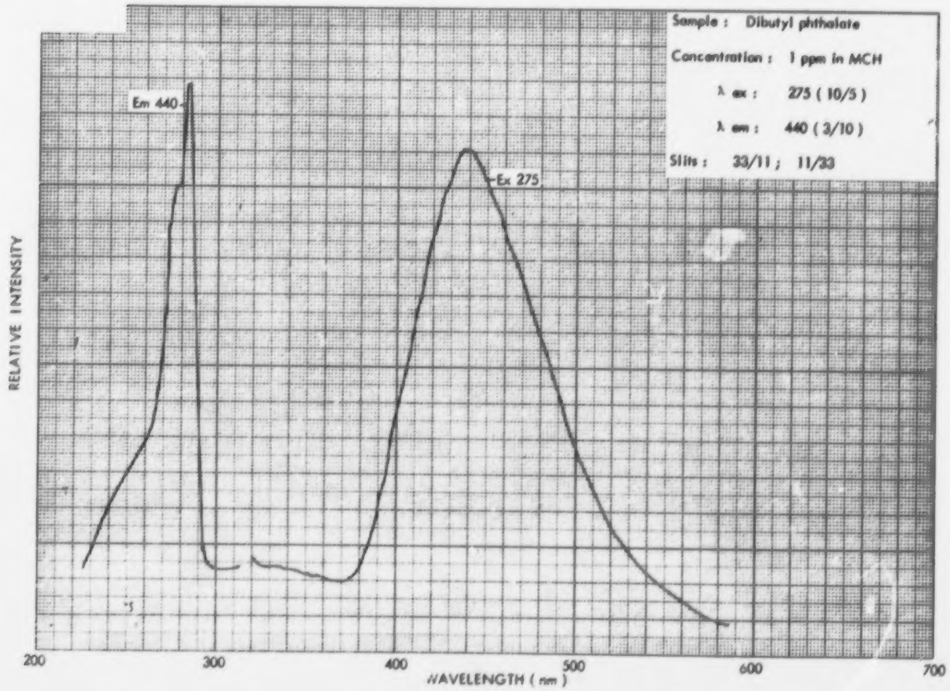


FIGURE 38. DIBUTYL PHTHALATE, 1 PPM IN MCH, 77°K

SECTION IX

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SECTION X

APPENDIX

METHOD FOR ESTIMATION OF PCB/DDT IN WATER

The method described herein can be summarized briefly as follows:

Grab samples of water are extracted with dichloromethane and the combined extracts concentrated nearly to dryness using a rotary evaporator (or the equivalent). The residue is then diluted with methylcyclohexane and the luminescence analyzed at 77°K. Identification and quantitation is based upon comparison of the observed spectra with those of Aroclor/pesticide standards. Since the analysis of water samples doped with these compounds may aid in the interpretation of the results (or in testing possible method modifications), these procedures are also described.

1. Equipment and Chemicals

The fluorescence instrumentation and accessories required are:

SF-100 Fluorispec.

X-Y recorder and graph paper.

Optical dewar and dewar positioner.

Suprasil quartz sample tubes (one or two dozen) with caps (caps can be fashioned as described in Section V of the main text).

Liquid nitrogen. The optical dewar capacity is about 40 cc and will hold nitrogen for one to two hours; this is usually adequate to complete four to eight analyses. A glass storage dewar is useful for refilling the optical dewar.

It is strongly recommended that the user become familiar with the basic instrument as described in the Fluorispec User's Manual. In particular, the user should practice obtaining spectra of common fluorescent compounds such as anthracene or quinine sulfate at room temperature before attempting the low temperature analyses described here.

Equipment and chemicals used for the collection and treatment of water samples are:

Collection bottles. Wide mouth glass bottles of 2 to 4 liter capacity are recommended. These should have screw-type lids with Teflon liners.

Magnetic stirrer. Several Teflon-covered stirring bars are also required.

Rotary evaporator (with flasks). If desired, a Kuderna-Danish concentrator can be substituted. In either case, the device should have a capacity of at least 100 ml.

Glass pipette, 50 ml, and pipetting bulb. This or an equivalent device is used to remove the solvent extract from the water sample.

Erlenmeyer Flask, 125 ml. This or an equivalent glass container is used to stir the extract with sodium sulfate. If the container volume is calibrated, it can also be used to deliver the extraction solvent to the water sample.

Screw-cap glass vials, 4 dram. These are used for final sample storage, and should be equipped with Teflon cap liners.

Sodium sulfate, anhydrous, reagent grade. This is used to remove traces of water from the extract.

Solvents. These are discussed in the next section.

Silica gel, activity grade I, 200 mesh. This is used for solvent purification.

2. Solvents and Purification

Methylcyclohexane (MCH)--Spectroquality grade or an equivalent purity should be used. This solvent generally contains trace amounts of toluene as an impurity, which can be removed by column chromatography using silica gel (see Section V). Using the slit combinations suggested in Section 3 of this Appendix, the purified solvent should be free of background luminescence at 77°K in the spectral region 300-500 nm when excited between 220 and 290 nm.

Dichloromethane (DCM)--Spectrograde material or an equivalent purity should be used although, since the boiling point is quite low (42°C), it may be possible to adequately purify poorer grades of material by distillation. To check the purity, 100 ml of this solvent should be evaporated nearly to dryness and then diluted with 5 ml of purified MCH. This solution is then analyzed for contaminants in the manner described for MCH. If necessary, this solvent can be purified by distillation.

Ethanol (95%, 5% water)--This is a useful solvent for doping PCB's and DDT-type compounds into water. This solvent, like MCH, should be checked for luminescence at 77°K. If necessary, the material should be distilled.

3. Intensity Standard and Instrument Optimization

Gain Linearity--The linearity of the SF-100 gain should be checked using an appropriate standard. In this case, a water Raman band is satisfactory (see User's Manual, Section II and Figure 5). With the recorder gain at the appropriate value (e.g., 0.1 volt/cm), the Raman peak is recorded at a fine gain of 10, the coarse gain being varied from 1 to 1000. The fine gain is checked by keeping the coarse gain fixed and recording the Raman band at the ten positions of the fine gain. If the instrument used in this study is typical, the coarse gain should be linear except for setting number 1, which gives a gain about 20% above the expected linear value. The fine gain is very non-linear, and in fact appears to be nearly quadratic. It is often desirable to use fine gain settings of either 5 or 10, where the gains differ by about a factor of two.

Low Temperature Standard--Spectra of a standard should be run daily to monitor instrument sensitivity. Since the instrument is a single beam type, long-term deterioration of the lamp and optics will result in a loss of intensity. Use of a standard thus allows the correction of instrument response to compensate for possibility sensitivity variations. The standard tentatively recommended is a 10 ppm solution of p,p'-DDT in MCH. This solution has been checked for photodecomposition at 77°K, exciting at the two principal excitation bands near 240 and 275 nm, with maximum slit widths

(33 combination). A nearly exponential decay was found at both wavelengths, and the decay rates appeared nearly identical. The emission intensity fell by 10% of its initial value after five hours, which is adequate time for about 50 complete excitation/emission scans (or 50 days if the procedure were done daily). However, since the volume irradiated is only about one-tenth of the total (0.5 ml) sample volume, the same solution could probably be used for even longer periods without significant decomposition.

About 0.5 ml of this solution is transferred to a quartz sample tube. If possible, the solution should be degassed in a clean vacuum system using several pump-freeze-thaw cycles and the tube sealed under vaccum. If this is not possible, the tube can be capped in the usual way. Since MCH slowly diffuses through the rubber cap, it is necessary to replace the solution in the tube every two or three weeks.

After the dewar positioner and optical dewar are installed and the emission wavelengths set on the graph paper (Section III of the User's Manual), the dewar is filled with liquid nitrogen, and the standard solution is lowered into the dewar. Lowering the sample completely into the dewar should require about 30 seconds. The frozen sample should be perfectly transparent with no cracks. (The sample can be observed shile in the dewar by opening the small door on the sample compartment.) If cracks do appear, the sample should be removed quickly and the solvent warmed with the fingers from the top down. (If the sample warms from the bottom, confinement of the warming solution by frozen material above it can result in breakage of the quartz tube.) The tube is then lowered again until a clear glass is obtained. If several attempts fail to produce a glass, the tube or solvent may contain water. If this happens, the tube should be emptied and flushed throughly with dry nitrogen gas before refilling (or a dry tube substituted). It may also help to keep light from striking the sample during the freezing process. This can be done by pulling up on the lever on the SF-100 labeled Source Filter (which inserts a pyrex glass plate into the optical path) and setting the excitation monochromator to 270 nm or lower.

Optimizing Dewar Position and Lamp Intensity--The slit combination 33/11 is used for the emission spectrum. Discussions of this notation are given in Section V and also in the User's Manual. The recorder vertical gain is set at 0.1 volt/inch and the time constant is set at 0.3 seconds. The excitation monochromator is set at 275 nm and the emission monochromator at 400 nm. The emission wavelength is then moved manually to shorter wavelength until the recorder pen begins to rise due to scattered exciting light (about 290-300 nm). With the room lights off, the knob on the dewar positioner which affects forward and backward movement of the dewar is adjusted until the scattered light intensity is minimized (there will probably be two such positions; the one giving least scatter is selected).

After minimizing scatter, the emission monochromator is then returned to the maximum of the DDT phosphorescence near 400 nm. The instrument gains are adjusted such that the intensity is brought to about 2/3 of the height of the paper. The Lateral Adjustment and Focus Controls for the xenon arc (see User's Manual, Figure 3) are then turned until the emission intensity is maximized; the control nearer the operator should be more influential in this respect. If these adjustments cause the pen to move off the chart paper, the instrument gains should be reduced accordingly. Proper execution of this procedure will result in minimum scattered light and maximum source intensity.

Running the Standard--The emission spectrum should then be scanned in the 300-500 nm region using the "slow" scan rate (1 nm per second). The pen will go off scale near 500 nm since the emission monochromator is now sensing the second order of the 275 nm exciting light. An appropriate filter (such as Corning 0-53 or equivalent) can be used on the emission side of the monochromator to eliminate this if desired; otherwise, the scan should be stopped when this point is reached. If the filter is used, the instrument fine gain can be increased slightly to compensate for the loss of intensity. The excitation spectrum is obtained next, monitoring at about 400 nm using an 11/33 slit combination. The excitation spectrum can be placed on the same page as the emission spectrum, but the wavelength calibration should be checked out and reset if necessary. The coarse gain setting will now have to be reduced to maintain equivalent peak height. This spectrum is then scanned from 220 to 300 nm. Analyzing slits used for these spectra are the narrowest available and give a spectral resolution of about 2 nm.

A record should be kept of relevant instrumental parameters, such as the wavelengths, slit combinations, recorder and instrument gains, and time constant.

4. Standard Solutions in MCH

The recommended standard solutions should consist of p,p'-DDT and Aroclors 1016, 1248, and 1254; however, other Aroclors, pesticides, etc., could be added.

Stock solutions at nominal concentrations of 100 ppm are prepared by dissolving 1 mg of the appropriate standard in 10 ml MCH. Successive ten-fold dilutions are prepared by combining 1 ml of a given concentration with 9 ml of solvent. The basic standard solutions should range from 100 to 0.01 ppm in MCH.

The glass containers used for storage of these solutions should have screw-type caps; a convenient size is 4 dram, which has a capacity of about 15 ml. The caps should be provided with Teflon liners to prevent possible contamination by cap materials.

Luminescence Analysis--It is suggested that the quartz sample tubes be checked for possible contaminants by filling them with MCH and exciting with the wavelengths recommended below. The tubes should then be filled to a depth of 7 to 8 cm (about 0.5 ml) with the appropriate standard solution and capped. It is perhaps best to begin with the highest concentrations in order to form a clear understanding of the nature of the luminescence signatures. The quartz tube is lowered into liquid nitrogen contained in the optical dewar. The rate of freezing should be similar to that described in the previous section and the frozen sample should be free of cracks.

With a piece of graph paper properly positioned on the recorder, the recorder vertical gain is set at 0.1 volts/inch, and the Fluorispec time constant is set at 0.3 seconds. The emission spectra should be run first, using the slit combination 33/11. The instrument gains should be adjusted to bring the peak of the emission to nearly the full height of the chart paper. The arc adjustment controls should be checked as described in the previous section to insure that the intensity is maximized. The emission monochromator is moved manually to shorter wavelengths until the pen begins to rise in response to the excited light. The emission spectrum is then scanned ("slow" speed)to longer wavelengths until the exciting light is sensed in second order. The scan should be stopped there unless the exciting light is filtered out. Similar instrument settings are used to record the excitation spectra, except the slit combination 11/33 is used; the instrument gain will have to be reduced if peak heights approximating those of the emission spectra are desired.

The wavelengths suggested for emission and excitation of DDT-type compounds and Aroclors are given below:

	Emission	Excitation
DDT	275	380
Aroclor	275, 290	400, 440, 470

It should be noted that excitation spectra monitored at 440 and 470 nm will show peaks near 220 and 235 nm, since the emission monochromator is sensing these excitation wavelengths in second order. These peaks can be eliminated by use of a filter as discussed in Section 3 of this Appendix.

Spectra of DDT and several Aroclors appearing in the main text may be used for comparison. Small wavelength differences (± 2 nm) may be found from those given here, and relative intensities may show some variation due to differences in lamp brightness and spectral distribution. Since the composition of Aroclors are somewhat variable, spectra obtained of other samples may show intrinsic differences.

Reduction of Data--Intensities should show a nearly linear dependence on concentration for concentrations below 10 ppm. Ideally, the integrated band intensities (areas) should be obtained; this, however, can be a very time-consuming process. Instead, peak maxima are used as an approximation to areas. Either emission or excitation peaks could be measured, but excitation peaks appear to be ultimately more useful in the analysis of environmental samples where background emission occurs. In general, the strongest (uncorrected) excitation band is selected for measurement. For DDT, this is the sharp origin band near 278 nm. The peak heights are measured relative to the underlying background, which should be due primarily to scattered light at low concentrations. The nature of this background can be determined by comparison with the pure solvent (MCH) spectrum at the same wavelengths and gain settings. The background should rise smoothly and continuously as the scatter peak is approached.

Band heights are conveniently measured in millimeters using the chart paper grid if desired. These are converted to approximate intensities by dividing the peak height by the product of the Fluorispec coarse and fine gain reading. This of course presumes that the gain settings are strictly linear over the range selected; if this is not the case, corrected gain values as discussed previously must be used. If the recorder gain has been varied, this gain must also be included. Finally, changes in the arc intensity as reflected in the intensity of the standard (Section 3 of this Appendix) should also be accounted for in the intensity determination. This is most easily accomplished by using the ratio of sample peak height to the peak height of a standard as a measure of normalized intensity:

$$I_x = \frac{H_x}{H_s} \cdot \frac{G_s}{G_x}$$

where

I_x = normalized sample intensity.

H_x = sample peak height measured at a gain of G_x.

H_s = standard peak height measured at a gain of G_s.

G_x, G_s = linearized instrument gain settings for measuring sample and standards respectively. In general, G is a product of the instrument coarse and fine gain setting, and would also include the recorder gain if this is varied.

The normalized sample intensity will then depend only on the sample concentration, independent of overall instrument sensitivity.

5. Doped Water Samples

Although this section is optional, it is recommended for several reasons. First, it enables the analyst to become familiar with extraction and concentration techniques prior to studies of environmental samples. Alternative extraction solvents or other new techniques may be evaluated with respect to recoveries of the compounds of interest. Finally, potential sources of contamination are more easily uncovered.

Water samples used initially should be distilled or of comparable purity. Purity can be checked by first extracting and analyzing an undoped sample. Doped samples of natural water can be analyzed in a similar fashion. The recommended procedure discussed below assumes a one-liter volume of water.

Dopant Solutions--Solutions of Aroclor and DDT compounds are prepared in purified ethanol at concentrations of 1, 10, and 100 ppm. Microliter pipettes are used to deliver the desired volumes into the water sample. The doped water sample should be stirred thoroughly using a Teflon or glass covered stirring bar activated by a magnetic stirrer.

Extraction and Concentration of Extracts--Two successive 75 ml aliquots of purified DCM are added to the bottle and the mixture stirred for at least an hour on the magnetic stirrer. Stirring speed should be fast enough to break the DCM layer into a fine emulsion dispersed throughout the water.

The combined extracts are dried with 10 to 20 grams of anhydrous Na_2SO_4. This can be done by stirring the extract with the drying agent in a small flask or beaker for about fifteen minutes. If the entire mass of desiccant becomes caked (hydrated), additional Na_2SO_4 should be added. The dried DCM is then transferred to a round bottom flask and brought almost to dryness using a rotary evaporator or other evaporative concentrator. The reduced pressure required for the rotary evaporator may be provided by a water aspirator. At 77°K, residual amounts of DCM in MCH cause the normally clear MCH glass to become slightly hazy, which contributes to scatter. For this reason the final DCM volume should be kept on the order of 0.1 ml.

The flask containing the DCM residue is then thoroughly rinsed with 5 ml MCH and the MCH solution stored in a screw-cap vial. This solution is ready for luminescence measurements.

Analysis--Low temperature luminescence analysis is performed in the manner described for the standard solutions in MCH. Measured peak heights are converted to approximate relative intensities as discussed in the previous section and the concentration in MCH determined from the appropriate analytical curves. Concentrations in water are obtained by multiplying the concentration in MCH by the ratio of the volume of MCH to that of the water sample.

6. Environmental Water Samples

Known sample volumes of two to four liters should be collected in glass bottles with Teflon-lined screw cpas. These should have been previously rinsed several times with purified DCM to remove residual impurities. Approximately 2.5 ml of concentrated HCl per liter are added to the sample as soon as possible after collection. The water sample is extracted with DCM and the extracts dried and concentrated as described in Section 5 of this Appendix. Five ml of purified MCH are added to the combined extracts and the resulting solutions are ready for luminescence analysis.

Luminescence Analysis--Luminescence analysis is performed using the instrumental parameters discussed in Section 4. Recommended wavelengths (in nm) are:

Emission: 275, 290

Excitation: 380, 400, 440, 470

The emission wavelengths are sufficient to excite both Aroclors and DDT derivatives. The excitation wavelengths 380 and 400 will produce little Aroclor excitation but will result in nearly maximum DDT intensity. Excitation spectra monitored at 440 and 470 nm will show reduced DDT intensity but nearly maximum Aroclor intensity.

For identification, spectral signatures should be compared with those of the Aroclors and DDT compounds in MCH. Better still, they should be compared with signatures obtained from natural water samples doped with these compounds, since natural water will contain a high background emission in the Aroclor/DDT region.

It is likely that this background emission will obscure the broad emission signatures of the Aroclors and DDT when present at low ppb levels in environmental waters. Since the excitation spectra of the Aroclors and DDT are much sharper than the natural background absorption, lower concentrations are observable in the excitation spectra. For this reason, quantitation is based upon excitation peak heights measured relative to the background absorption, which should be nearly continuous in the region of interest.

Reduction of Data--Peak heights are converted to relative intensities and thus to concentrations as described in Section 4. The value obtained would be the actual value if 100% of the material were recovered. The actual recoveries will probably be less than this. If recoveries have been estimated from doped water samples, the concentration obtained here can be divided by the fraction recovered.

7. Accuracy, Sensitivity, and Possible Interferences

The accuracy and precision of the determination are influenced by both the luminescence measurement and by the extraction procedure. These sources of error are discussed in detail in Section VII of the main text. For the analysis of natural water samples for which recoveries have been determined, the maximum percentage error should be in the range of 25-40%. The estimated precision of the determination is approximately 25%.

The detection limits for DDT and Aroclor 1254 in MCH at 77°K should be approximately 0.03 and 0.002 ppm using the recommended instrument parameters. Similar sensitivities are expected for DDD and the more highly chlorinated Aroclors. Since these values were determined using optimum resolution (2 nm), lower limits might be achieved using wider analyzing slits, but this would result in loss of resolution and therefore specificity.

Detection limits for DDT and Aroclor 1254 in one-liter samples of pure water should be approximately 0.5 and 0.03 ppb respectively using the extraction and spectral method described. For natural waters rich in dissolved fluorescent organic matter and suspended particulates, detection sensitivities may be from 10 to 100 times higher depending on the extent of adsorption on particulates and the fluorescence background level.

Phthalic acid esters (phthalates) may be encountered both in water samples and as laboratory contaminants. Since the phosphorescence excitation/emission spectra may mimic certain Aroclor spectra (and thus interfere), it is strongly recommended that spectra be obtained of several of the more commonly used phthalates, such as dibutyl phthalate and di-2ethylhexyl phthalate. Excitation spectra of phthalates should show less variation in structure than Aroclors when monitored at several different emission wavelengths, and this feature should permit some discrimination. However, at the present time phthalates should be regarded as potential interferences for Aroclors.